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PRIMARY RHEGMATOGENOUS RETINAL DETACHMENT: CLINICAL EPIDEMIOLOGY AND GENETIC AETIOLOGY

Danny Mitry BA MB BCh BAO MRCSI (Ophth)

A thesis submitted to the University of Edinburgh for the degree of Doctor of Philosophy in the college of Medicine & Veterinary Medicine

2012

Declaration

I confirm that this thesis has been composed by me and that the work is my own. I am a member of a research group. I have clearly indicated where work was conducted by other members of the research group. I confirm that this work has not been submitted for any other degree or professional qualification.

Name:__ Danny Mitry_____ Date:__ 1st August 2012_

Abstract

Primary rhegmatogenous retinal detachment (RRD) is one of the most common ophthalmic emergencies. RRD is caused by a full thickness break in the retina which initiates separation of the neurosensory retina from the underlying retinal pigment epithelium. The subsequent accumulation of fluid within this potential space extends the area of detachment and causes visual loss.

Previous assessments of RRD incidence have demonstrated large differences in case definition and methodology, with incidence estimates varying 3-fold geographically and in different time periods. To date there have been no systematic or prospective incidence estimates of primary RRD in the U.K. In this thesis I present the findings of a 2-year epidemiology study that prospectively aimed to recruit all incident cases of primary RRD diagnosed in Scotland. Case recruitment from consenting participants comprised a detailed questionnaire and a blood sample. In this thesis, I present the findings of the Scottish retinal detachment study that examined the incidence, demographic features, temporal incidence trends, as well as clinical and socio-economic associations of primary RRD in Scotland. From the clinical and genetic resource I assembled, I calculated the first population based estimate of the sibling recurrence risk ratio for RRD and designed and assisted in the analysis of the first case-control genome wide association study of this condition.

Results from this study have estimated the annual incidence of primary RRD in Scotland to be 12.05 per 100,000 population. Based on this estimate, there are approximately 7,300 new cases annually in the United Kingdom. RRD incidence increases with age, is more common in men and right eyes, and is strongly associated with socio-economic affluence. In addition, using

hospital episode data, the overall age-standardised incidence of RRD in Scotland was shown to be steadily increasing since 1987 with an average annual increase of 1.9%.

Analysis of the clinical findings highlighted that the majority of RRD cases are caused by more than one retinal break; an important consideration for appropriate surgical management. Ocular trauma, previous cataract surgery, family history, and retinal degeneration are important predisposing features. In addition, over a 2 year period approximately 7% of individuals will suffer a RRD in the fellow eye representing an important risk of bilateral visual loss.

Furthermore, I demonstrate that the risk of having an affected sibling with RRD is increased 2-fold given that one sibling has had the condition, substantiating a genetic component to the pathogenesis of this condition. In the final aspect of this thesis I will present the design and analysis of a two stage case-control genome-wide association study examining the role of common genetic variants and selected candidate genes in predisposing to RRD development.

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COMMON ABBREVIATIONS

1958BC	U.K. 1958 Birth Cohort
AAPC	Average annual percent change
CDCV	Common disease common variant theory
CSR	Cataract surgery rate
DNA	Deoxyribonucleic acid
EM	Electron Microscopy
FEVR	Familial exudative vitreoretinopathy
GAG	Glycosaminoglycan
GRT	Giant retinal tear
GWAS	Genome Wide Association Study
HA	Hyaluron
HST	Horse shoe tear
HWE	Hardy-Weinberg Equilibrium
IBD	Identity by descent
IBS	Identity by state
ILM	Inner limiting membrane
IPM	Interphotoreceptor matrix
LD	Linkage disequilibrium
MAF	Minor allele frequency
MEH	Moorfields Eye Hospital
MH	Macular hole
NSR	Neurosensory retina
PCR	Polymerase chain reaction
PPV	Pars plana vitrectomy
PVD	Posterior vitreous detachment
PVR	Proliferative vitreoretinopathy

QQ plot	Quantile-quantile plot
RH	Round hole
RPE	Retinal Pigment Epithelium
RRD	Rhegmatogenous retinal detachment
SER	Spherical equivalent refraction
SNP	Single nucleotide polymorphism
SOCCS	Scottish Colorectal Cancer Study
WTCCC	Wellcome Trust Case Control Consortium
WTCRF	Wellcome Trust Clinical Research Facility Edinburgh

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LIST OF PRIZES AND PUBLICATIONS FROM THIS WORK

PRIZES AWARDED

Barbara Knox Medal and research award (E1,000) for best overall presentation

Irish College of Ophthalmologists bicentennial conference

Eyecare Charity Research Award- 500 GBP

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ORAL PRESENTATIONS

May 2011
Population based estimate of the sibling recurrence risk ratio for rhegmatogenous retinal detachment
Association of Research and Vision in Ophthalmology (A.R.V.O), Ft Lauderdale

Nov 2010
Early post-operative outcome: results from the Scottish retinal detachment study British and Eire Association of Vitreo-retinal Surgeons (B.E.A.V.R.S) Newport, Wales

Nov 2010
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Nov 2010
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The epidemiology of retinal detachment in Scotland, New England Ophthalmological Society and the Irish College of Ophthalmology Annual Conference, Dublin

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Epidemiology and Clinical characteristics of the first 500 cases, B.E.A.V.R.S, Reading

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Is retinal detachment associated with affluence?, B.E.A.V.R.S, Reading

Nov. 2007
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The epidemiology of retinal detachment in Scotland, Royal College of Ophthalmology Annual Congress, Liverpool

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Introduction

Primary rhegmatogenous retinal detachment (RRD) is the most common ophthalmic emergency. RRD is caused by a full thickness break in the retina which initiates separation of the neurosensory retina from the underlying retinal pigment epithelium. The subsequent accumulation of fluid within this potential space (the subretinal space) extends the area of detachment and causes visual field loss.(D'Amico 2008) Most cases of RRD present when the macula is affected and require intervention to restore vision or prevent further visual loss. The mainstay of RRD treatment is surgical, accounting for an important proportion of ophthalmic hospital in-patient admissions at an annual cost of over £1.3million in the U.K.(Patel *et al.* 2004)

Previous assessments of RRD incidence have demonstrated large differences in case definition and methodology, with incidence estimates varying 3-fold geographically and in different time periods. To date there have been no systematic or prospective incidence estimates of primary RRD in the U.K. In addition, despite a known familial component to RRD, there have been no published genome-wide association studies on RRD. Common genetic variants which predispose to developing this common and potentially blinding condition are therefore unknown.

This thesis presents the findings of a 2-year epidemiology study that prospectively aimed to recruit all incident cases of primary RRD diagnosed in Scotland. Based on this clinical and genetic resource my aim was to calculate the incidence of primary RRD, to explore clinical associations within the study group, and to plan and conduct a 2 stage genome wide association study.

CHAPTER 1- Anatomical considerations

1.1 The retina

The retina contains the receptors for vision and is the innermost layer of the eye. It functions to transform visual light stimuli into neural signals which are transmitted and interpreted in the brain. The retina is bounded externally by Bruch's membrane and the choroid and internally by the vitreous. It is a transparent membrane being thickest at the posterior pole (0.56mm) and thinning towards its anterior extension, (0.1mm) the ora serrata. It is continuous posteriorly with the optic nerve and anteriorly with the epithelium of the ciliary body. (Snell R & Lemp M 1998) It consists of two layers, an outer pigmented layer (RPE) and an inner neurosensory layer (NSR) between which there is a potential space important in rhegmatogenous retinal detachment pathology, the sub-retinal space. Based on light microscopy and principally for descriptive purposes, the retina consists of ten layers. These are designated (from posterior to anterior): The retinal pigment epithelium (RPE), the rods and cones, the outer limiting membrane, the outer nuclear layer, the outer plexiform layer, the inner nuclear layer, the inner plexiform layer, the ganglion cells, the nerve fibre layer and the inner limiting membrane (ILM). The outer nuclear layer contains the nuclei of the rods and cones and the inner nuclear layer contains the nuclei of the horizontal cells, bipolar cells, Muller cells and amacrine cells. The outer plexiform layer contains the synapses between the rods and cones and the bipolar, horizontal and amacrine cells. The inner plexiform layer is contains the synaptic connections of the bipolar, amacrine and ganglion cells. The ganglion layer consists of the nerve fibres of the

ganglion cells converging towards the optic disc. Muller cells are neuro-glial support cells abundant in the retina. They have long processes and that extend radially across the retina. The outer limiting membrane is made up of a layer of zonulae adherents between the photoreceptor cells and the radial processes of the Muller cells. At the vitreous surface, the Muller cells have expanded terminations covered by a basement membrane. This layer forms the retinal aspect of the vitreo-retinal interface, which has an important role in vitreo-retinal pathology and is known as the inner limiting membrane. (Michels RG *et al.* 1990; Forrester *et al.* 1996)

1.1.1 The cells that limit the sub-retinal space

The NSR contains the photoreceptors and nerve fibre layer. The RPE is a single layer of cells extending from the optic nerve head to the ora serrata which have numerous functions in metabolism, photo-transduction and adhesion to the neurosensory layer. Between these two layers is a potential space, the sub-retinal space. When the retina detaches, the sensory retina separates from the RPE, and the RPE remains attached to Bruch's membrane and the underlying choroid.(Michels RG *et al.* 1990)

1.1.2 Photoreceptors

Photoreceptors are one of three principal neuron cells types (along with ganglion and bipolar cells) in human retina responsible for phototransduction and the relay of impulses generated by light. In the human eye, two types of photoreceptor predominate: rods and cones, which are situated in the outer aspect of the neurosensory retina. There are approximately 115 million rods and 6.5 million cones in the human retina. Rods are responsible principally for sensing contrast, brightness and motion, whereas cones function to sense fine resolution, spatial resolution and colour vision. The density of rods and cones varies topographically. The peripheral retina is rod-

dominated with an average density of 30,000 rods per mm^2. The cone density increases towards the macula (150,000mm^2) and the fovea contains cones only.

Anatomically rods and cones consist of a long slender cell with an inner and outer segment joined by a connecting stalk. The inner segment joins with the cell nucleus and nerve fibre axons which pass into the outer-plexiform layer of the retina where they form synaptic terminals (cone pedicle or rod spherule) with bipolar cells and other inter-neurons. The outer segments of rods and cones are different in structure anatomically and in function physiologically but they both contain the visual pigments responsible for light absorption and for initiation of a neuro-electrical impulse.(Forrester *et al.* 1996)

Rod Cells

Rods are long (~100µm) cells whose outer segment contains a visual pigment, rhodopsin within membrane bound lamellae or disks (up to 1,000 per cell and 10-15nm thick) that are enclosed by a single membrane. The disks are formed at the base of the outer segment and over a period of 10 days they travel to the tips of the outer segment where they are phagocytosed by the microvilli of the RPE in a circadian manner. The rods are separated from the RPE by a modified extra-cellular ground substance called the inter-photoreceptor matrix.

Cone Cells

Cones are shorter than rods (~70µm) and are usually divided into three types depending on the wavelength of maximal sensitivity (blue, green, red corresponding to short, medium and long wavelength). Cone outer segments tend to be shorter than rods and conical in shape. The lamellae in cones are

not surrounded by a membrane as in rods, but are in free communication with the inter-photoreceptor matrix. Cones have a longer lifespan than rods, they are not produced in the same manner as rods and do not undergo phagocytosis by the RPE.(Forrester *et al.* 1996)

1.1.3 Retinal pigment epithelium

The RPE is a monolayer of columnar/cuboidal epithelial cells extending from the margin of the optic nerve head to the ora serrata, where it is continuous with the pigment epithelial layer of the pars plana. The basal aspect of the RPE lies on Bruchs membrane and the apical surface is intimately associated with the photoreceptor outer segments. The number of RPE cells in each eye is approximately 4.2-6.1 million. The RPE has many functions which are critical in maintaining normal visual function. (Forrester *et al.* 1996; Green & Sebag 2006)

1.1.4 Geographic regions of the retina

The clinical and anatomical structure of the retina is clearly defined into several topographic regions (See Figure 1.1) (Michels RG *et al.* 1990):

- The posterior pole (macula or area centralis)(blue circle): This is a 5-6mm diameter circular zone of retina situated between the superior and inferior temporal retinal arteries. This region is cone dominated.

- The fovea (yellow circle): This is a 1.5mm diameter area in the posterior pole, 3mm lateral to the optic disc. It is partly yellow due to carotenoid pigments contained in the cone axons. The foveal region is on the optical axis, so that light fixed by an observer falls directly on the fovea.

- The fovea centralis (foveola) (red circle): This is a 0.35mm area in the centre of the macula consisting of a depression surrounded by slightly

thickened margins. Cone receptors are maximally concentrated here. The foveola is avasular and depends entirely on the underlying choriocapillaris for nutritional support.

- The peripheral retina is the remainder of the retina outside the posterior pole. The distance from the optic disc to the ora serrata is 23-24mm temporally and 18.5mm nasally. The peripheral retina thins as it extends anteriorly and possesses only one layer of ganglion cell bodies.

- The ora serrata is the dentate-like anterior margin of the NSR. At this transition region, the NSR is continuous with the layers of the ciliary body epithelial cells. The ora serrata extends 1-2mm more anteriorly on the nasal side.

  For descriptive purposes, the retina is divided into nasal and temporal halves by a vertical line through the fovea. The optic nerve head is used as a central point to describe the four quadrants of the retina – supero-temporal, supero-nasal, infero-temporal and infero-nasal. The area of the human retina is 1250mm^2.

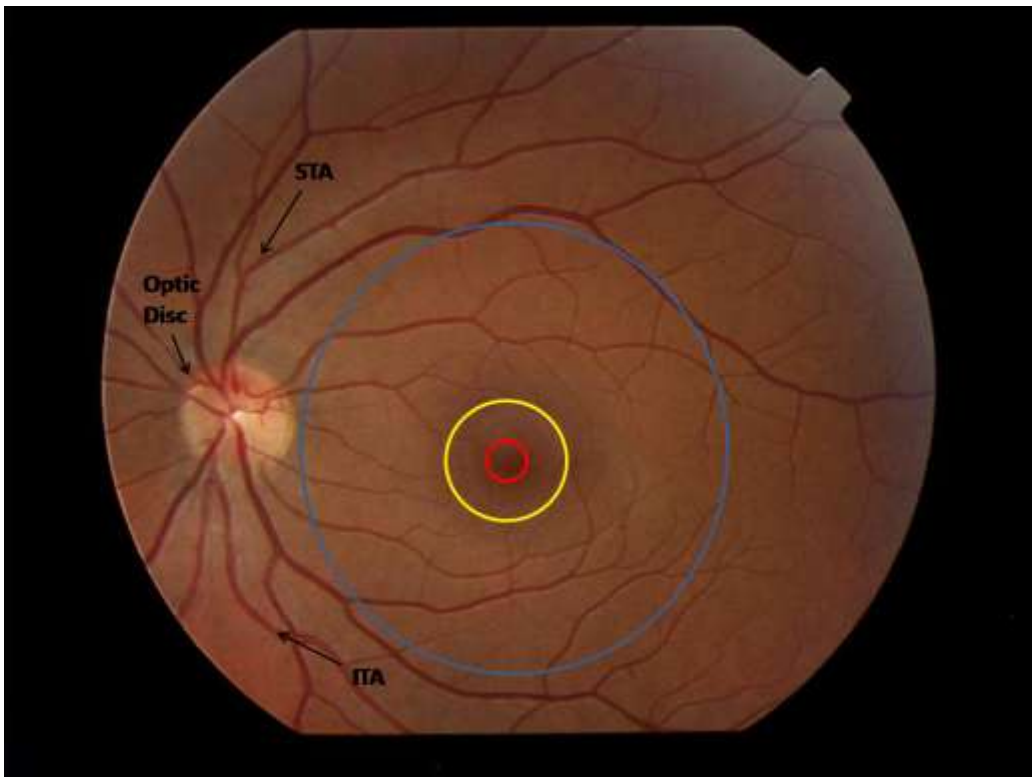


Figure 1.1 – Illustrating the geographic features of the central retina- Macula(blue circle); fovea (yellow); foveola (red). STA- Superior temporal artery; ITA- Inferior temporal artery

1.2 The vitreous

Normally, the vitreous is a homogenous gel-like structure, with several distinct anatomical regions including the vitreous body, the vitreous base and the vitreous cortex.(Michels RG *et al.* 1990) The central vitreous comprises the main bulk of the vitreous body and is traversed by an embryonic vascular remnant (Cloquet's canal), which extends posteriorly as the space of Martegiani present over the optic nerve head. Within the central vitreous, collagen fibrils are low in concentration and run in an antero-posterior direction inserting into the vitreous base anteriorly and the vitreous cortex posteriorly. Anteriorly, the vitreous gel is concave and is

attached to the posterior surface of the lens capsule (Weigert's ligament).(Green & Sebag 2006; Sebag & Balazs 1985)

The vitreous cortex is a thin layer of vitreous (100μm-300μm) surrounding the vitreous body. It may be distinguished from the vitreous body by the higher concentration of collagen fibrils. The anterior aspect of the vitreous cortex extends from the vitreous base anteriorly and across the posterior surface of the lens. The anterior limit is known as the anterior hyaloid face. This region is in direct contact with the aqueous humour providing a potential diffusion surface. The posterior vitreous cortex lines the surface of the retina behind the vitreous body. The vitreous cortex is absent over the optic disc and thinned over the macula. The fibrils of the cortex extend parallel to the retina and are thought to insert indirectly into the inner limiting membrane of the retina.(Le Goff & Bishop 2008; Foos 1972b; Balazs 1975)

The vitreous base is an annular zone, straddling the ora serrata. It extends 1-2mm anterior to the ora serrata and 3-4mm posterior to the ora serrata on the retina. The vitreous in this region is characterised by dense collagen fibrils which are strongly adherent to the anterior retina and the non-pigmented ciliary epithelium of the pars plana forming an unbreakable adhesion.(Foos 1972b; Bishop 2000)

1.2.1 Vitreous attachment

The vitreous is attached to all contiguous structures, but the firmness of attachment varies, which has important implications in vitreo-retinal pathology:

Vitreous base- The vitreous base is the firmest zone of attachment between the vitreous gel and surrounding structures. Collagen fibres in this region

have a radial orientation and are densely interwoven with basement membranes from the adjacent retinal and ciliary epithelial cells. Anterior to the ora serrata, the fibres insert directly into 'crypts' and invaginations in the retinal basement membrane or into the plasmalemma of Muller cells.(Foos 1972a) Posterior to the ora serrata, Foos originally described progressive changes in the structure of the vitreous base with age, where interdigitations develop between the collagen filaments of the vitreous base and the basal layer of the retina. This results in a gradual posterior migration of the posterior edge of the vitreous base.(OKUN 1961; Foos 1972a; TENG & CHI 1957) Vitreous base fibres inserting anterior to the ora serrata are known as the anterior loop fibres which play a role in anterior proliferative vitreoretinopathy membrane formation and transmission of traction to the peripheral retina and ciliary body.(Green & Sebag 2006)

Optic nerve head – The vitreous cortex is adherent to the periphery of the optic nerve head, with vitreous fibrils intermingling with thickened basement membranes of Muller cells. The adhesion at this site is strong in young people and weakens with age.(Michels RG *et al.* 1990; GRIGNOLO 1952)

Lens and Parafoveal area- The cortical vitreous is also moderately adherent around the posterior aspect of the lens capsule, the posterior pole and margin of the fovea.

Retinal vasculature - Firm attachments are also present to the retinal vessels in the mid-periphery. The attachment to these vessels accounts for the bleeding which can occur during a posterior vitreous detachment. The cortical vitreous may also be attached to the retina around other areas of pathology such as chorio-retinal scars, lattice degeneration and cystic retinal tufts.(Michels RG *et al.* 1990) Figure 1.2 highlights the anatomy of the vitreous and sites of vitreo-retinal attachment.

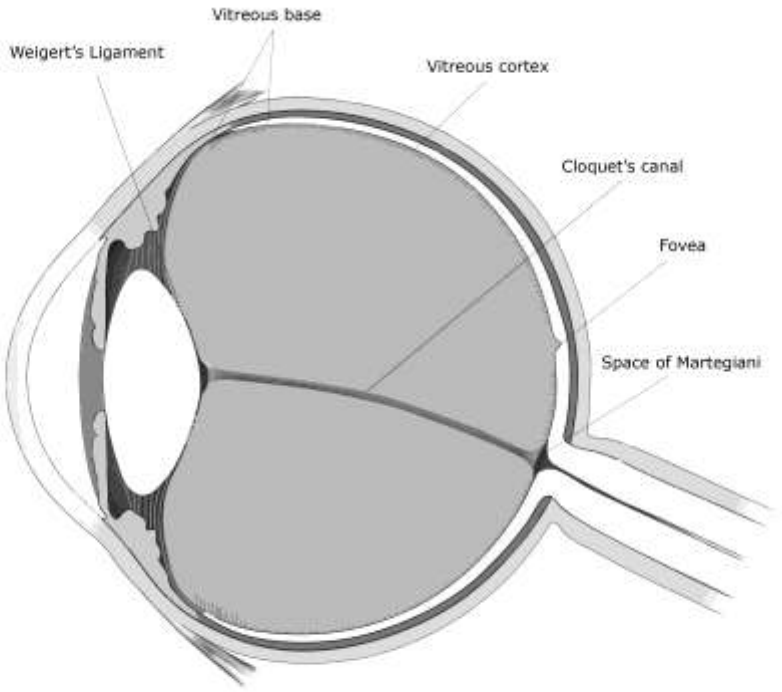


Figure 1.2 – Schematic diagram demonstrating the anatomy of the vitreous.

1.3 Vitreous biochemistry

The vitreous is an acellular extra-cellular matrix consisting mainly of water (98%) and contains macromolecules with complementary properties responsible for structure and function. The fibrillar macromolecules of an extra-cellular matrix provide the shape, strength and flexibility, and the charged carbohydrates, (principally gycosaminoglycans) by attracting water and counter-ions, provide a swelling pressure that spaces the fibrillar proteins and resists compressive forces. The principal fibrillar component providing structure to the vitreous is collagen and the principal glycosaminoglycan (GAG) is hyaluron (HA).(Bishop 2000; Le Goff & Bishop 2008)The collagen fibrils provide a scaffold-like structure that is 'inflated' by the HA.(Sebag 2004) If collagen is removed, the remaining HA forms a

viscous solution, if HA is removed, the gel shrinks but is not destroyed.(Comper & Laurent 1978)

1.3.1 Collagen

Collagen molecules are formed from 3 polypeptide (alpha) chains which fold in a characteristic triple helical formation. This consists of an amino acid repeat structure which requires that each 3rd amino acid residue is a glycine so that the configuration is (Gly-X-Y), where X and Y can be any amino acid. The presence of different amino acids allow for glycosylation or intra-molecular cross-linking. Vitreous consists of rope like structures composed of collagen type II, a hybrid of types V/XI and type IX collagen in a molar ratio of 75:10:15.(Bishop 2000; Le Goff & Bishop 2008; Sebag 2004)

Type II Collagen

This accounts for 75% of total collagen. It is secreted as procollagen with terminal extensions N-propeptide and C-propeptide. These terminal extensions are cleaved by specific enzymes which reduce the solubility of the collagen and allow it to participate in fibril formation. The N-propeptide undergoes alternative splicing at the mRNA level, where exon 2 is included (IIA procollagen) or is not (IIB procollagen). Type IIA predominates in adult vitreous, and has been shown to have properties in binding growth factors.(Bishop *et al.* 1994a; Bishop *et al.* 1994b; Zhu *et al.* 1999)

Type V/XI Collagen

Vitreous contains a hybrid of type V and type XI collagen. This type of collagen comprises between 10-20% of vitreous collagen and co-assembles with type II collagen to form the fibril core.(Le Goff & Bishop 2008) The N-terminal of type XI collagen is retained on the surface and plays an

important role in regulating fibril diameter. Collagen V it thought to be important in initiating collagen fibril formation.(Blaschke UK *et al.* 2000; Gregory *et al.* 2000; Wenstrup *et al.* 2004)

Type IX Collagen

Type IX collagen represents approximately 20% of vitreous collagen. It covalently links to the surface of the fibrils in a regular periodicity; however it cannot form fibrils in isolation. Type IX collagen is a non-fibrillar collagen and belongs to the family of fibril-associated collagens with interrupted triple helices, which also includes type XII, XIV, and XVI collagen. Type IX collagen can exist in a proteoglycan and non-proteoglycan form. In the vitreous it is synthesized in proteoglycan form.(Le Goff & Bishop 2008; Bishop *et al.* 1994a; Bos *et al.* 2001)

Type VI Collagen

Recently, Bishop et al have demonstrated that type VI collagen has been found in bovine and human vitreous. It is not thought to be a component of the collagen fibrils but forms separate micro-fibrils. It has also has a role in binding of fibrillar collagen and hyaluronan.(Bishop *et al.* 1996; Kielty *et al.* 1992) Figure 1.3ab illustrates the macro-molecular structure of the vitreous in the youthful and aged eye.

1.3.2 Non-collagen structural proteins

Fibrillin

Fibrillin containing microfibrils have been demonstrated in the vitreous. These are a family of glycoproteins present as isolated microfibrils in vitreous. (ie. They do not associate with the heterotypic collagen fibrils present in abundance.) They tend to be associated with elastin in many body

tissues, but this does not appear to occur in the vitreous. The major component of these microfibrilins in the eye is fibrillin-1, which contains a number of calcium binding epidermal growth factor-like domains. A mutation in the gene coding for fibrillin-1 (Ch. 15q21) causes Marfan syndrome, which carries an increased risk of RRD. However, it is not known if the changes in vitreous structure confer this increased risk or if the risk is related to other ocular phenotype features such as myopia and lens dislocation.(Bishop 2000; Le Goff & Bishop 2008; Mayne *et al.* 1991; Ren *et al.* 1991)

Opticin

Opticin is a leucine-rich repeat protein which has shown to be a dominant extra-cellular protein that binds the heterotypic collagen fibrils of vitreous. It is thought to be secreted by the non-pigmented ciliary epithelium and has been immunolocalised to the ILM, lens capsule as well as cortical and basal vitreous. Opticin may have a role in vitreo-retinal adhesion, associating with type XVII collagen/endostatin found at the ILM and it also binds growth hormone and several GAGs including heparin sulphate and chondroitin sulphate.(Le Goff & Bishop 2007; Sanders *et al.* 2003; Ramesh *et al.* 2004)

Other collagen binding macromolecules of unknown significance have also been found including VIT-1 and fibroncetin.(Mayne *et al.* 1999; Menasche *et al.* 1997)

Glycosaminoglycans

Glycosaminoglycans (GAGs) are extracellular molecules composed of long chains of repeating disaccharide units, all GAGs except HA are bound to a protein core (forming proteoglycans) and undergo varying degrees of modification. The vitreous contains HA and small amounts of chondroitin sulphate (CS) and heparan sulphate (HS).(Le Goff & Bishop 2008)

Hyaluronan

Hyaluronan (HA) is the predominant GAG in vitreous and is found in its highest concentration in the vitreous cortex. Its' concentration has been shown to increase with age, peaking at 20 years, before increasing again after 70 years. HA is a highly hydrated polyanion which forms space filling networks and has visco-elastic properties. HA chains can be long and can associate with several other chains through entanglement. The entangled gel-like nature of the polysaccharide is highly hydrophilic. This water binding capacity gives a high optical clarity to the vitreous and also prevents rapid movement of water into or out of the vitreous acting to resist large changes in the globe volume.(Le Goff & Bishop 2008; Foulds 1987; Sebag 2004; Balazs 1975)

Chondroitin sulphate

The vitreous contains two CS proteoglycans, collagen IX and versican. Of these, versican is present in large quantities. The C-terminal region of versican contains several domains and the N-terminal binds to HA supported by a link protein. By binding HA at its N-terminal, versican may also interact with several other components through its C-terminal. In has been shown that versican interacts with microfibrillar proteins fibulin-1 and fibulin-2. Mutations altering the CS domains of versican have been implicated in Wagner syndrome.(Bishop *et al.* 1994a; Miosge *et al.* 1998; Reardon *et al.* 1998; Le Goff & Bishop 2008; Bishop 2000; Kloeckener-Gruissem *et al.* 2006)

Heparan Sulphate

HS proteoglycans are major components of basement membranes and have been found on the ILM of the retina. HS is found in higher concentrations in

the vitreous during development and concentrations are very low post-natally.(Halfter *et al.* 2005; Le Goff & Bishop 2008)(Table 1.1)

Principal structural proteins in human vitreous			
	Composition	**Structural features**	**Distribution**
Collagens			
Fibrillar collagens			
Type II	[α1(II)]3	70% of vitreal collagen; type IIa predominates; binds growth factors TGF-β1 and BMP-2(Bishop *et al.* 1994a; Zhu *et al.* 1999)	Vitreous body and cortex
Type V/XI	[α1(XI)][α2(V)]	Co-assembles with type II forming fibril core; role in regulating fibril diameter and initiating fibril formation(Blaschke UK *et al.* 2000; Wenstrup *et al.* 2004)	Vitreous body and cortex
Fibril associated collagens			
Type IX	[α1(IX)][α2(IX)][α3(IX)]	Lateral association with type II; binds GAGs; role in maintaining regular fibril spacing(Bos *et al.* 2001; Bishop *et al.* 1994a)	Vitreous body and cortex
Type VI	[α1(VI)][α2(VI)]	Lateral association with type I, II and hyaluronan; may surround vitreous cortex and mediate adhesion between vitreous cortex and ILM(Bishop *et al.* 1996; Kielty *et al.* 1992)	Vitreous body, ILM
Type XVIII	[α1(XVIII)]	Potential role in anchoring vitreous matrix(Ponsioen *et al.* 2008)	ILM
Sheet forming collagens			
Type IV	$[\alpha1(IV)]_2[\alpha2(IV)]$	Forms a two-dimensional network; principal component of basement membranes(Ponsioen *et al.* 2008)	ILM
Non-collagen			
Fibrillin	Fibrillin-1 containing microfibrils	Contributes to vitreous structural support and elasticity(Mayne *et al.* 1991; Le Goff & Bishop 2008)	Vitreous body, Lens zonules
Opticin	Leucine rich repeat protein	Binds heterotypic collagen fibrils; role in assembly of collagen fibrils and preventing fibril aggregation(Le Goff *et al.* 2003; Le Goff & Bishop 2007)	Vitreous cortex, ILM

Glycosaminoglycans(GAGs) - Repeating disaccharide: D-glucuronic acid and N-acetyl-D-glucosamine			
Hyaluronan	Non-sulphated	Major vitreous component; highly hydrated; forms space filling network(Theocharis *et al.* 2008)	Vitreous body
Chondroitin sulphate	4-sulphation; 6-sulphation	Complexes with hyaluronan stabilising vitreous structure; interacts with ECM proteins(Theocharis *et al.* 2008; Bishop 2000)	Vitreous body
Heparan sulphate	Undergoes sulphation and acetylation	Potential role in ILM/vitreous cortex anchoring(Bishop 2000)	Vitreous cortex/ILM

Table 1.1 – The structural features and known vitreoretinal distribution of the principal structural components of the vitreous and vitreoretinal interface. (ECM- Extra-cellular matrix)

1.4 Vitreo-retinal adhesion

1.4.1 Vitreous cortex and inner limiting membrane

The basement membrane of the Muller cells forms the principal component of the inner limiting membrane of the retina.(Green & Sebag 2006) The ILM consists of 3 layers, whose thickness varies topographically and increases with age – the lamina rara, immediately adjacent to the Muller cells is 0.03-0.06μm thick and demonstrates no topographic or age related changes.(Malecaze *et al.* 1985) The middle layer, lamina densa is thinnest at the fovea and disc (0.01-0.1μm), remaining thin at the vitreous base and equator, but thickens at the posterior pole to 0.5μm to 3.2μm. The lamina rara externa is the innermost layer, contiguous with the vitreous cortex.(Foos 1972b; Foos 1974; Matsumoto *et al.* 1984; Sebag 1992) The vitreal surface of the human ILM is smooth, and the posterior aspect is irregular, filling the spaces formed by the underlying irregular membranes of

the sub-retinal glial cells. This feature is most marked at the posterior pole. At the rim of the optic nerve head, the thickened posterior ILM ends abruptly and continues as a thin basement membrane thought to be the basal lamina of the astroglial cells in the optic nerve head. (known as the inner limiting membrane of Elsching). Over the central portion of the optic nerve head, the membrane thins to 20nm and is composed of GAGs and no collagen. (known as the central meniscus of Kuhnt) The thinness and chemical composition of these areas are thought to account at least in part for the frequency of pathological proliferative changes and abnormal cell migration occurring around the optic nerve head. Similarly in the foveal area, the density of the Muller cell processes decreases significantly and the ILM becomes markedly attenuated (10-20nm thick).(Matsumoto *et al.* 1984; Russell *et al.* 1991; Sebag 1992; Anderson 1970; Heegaard *et al.* 1988)

Unlike the dense attachment at the vitreous base, collagen fibrils of the cortical vitreous do not appear to insert into the lamina rara externa of the ILM, but are orientated parallel to it.(Matsumoto *et al.* 1984)The particular mode of adhesion between the ILM and vitreous cortex remains unknown, but the unifying concept is that these two anatomically distinct entities adhere together via an extra-cellular matrix 'glue.'(Green & Sebag 2006)

Immunocytochemical studies have confimed that laminin, fibronectin and collagen type IV are present in human ILM. Collagen type XVIII has been identified in the chick and mouse ILM, the absence of which causes vitreoretinal separation in mice.(Halfter *et al.* 1998; Fukai *et al.* 2002) Type IV collagen forms a scaffold-like network that stabilises the ILM and laminin molecules form an independent scaffold and can interact with membrane receptors.(Timpl 1996; Timpl & Brown 1996) Fibronectin is present on the surfaces of most cells and in many extracellular matrices throughout the

body. It is an extracellular glycoprotein which binds with high affinity to interstitial collagens, proteoglycans, and HA.(Yamada & Olden 1978; Pearlstein *et al.* 1980) Laminin is restricted primarily to basement membranes and binds strongly to type IV collagen of epithelial cells.(Timpl *et al.* 1979) Topographic and age-related variation in the distribution of ILM constituents has been noted in human retina.(Kohno *et al.* 1987; Halfter *et al.* 2000) Immunoflourescent studies of the ILM have shown a bi-laminar pattern of fibronectin and laminin in thickened ILM at the posterior pole of older eyes. Young eyes did not demonstrate this bi-laminar pattern. This association also varies topographically, being weakest at the equator and strongest at the posterior pole. (Kohno *et al.* 1987) There was no correlation between the presence of laminin and fribronectin and the occurrence of posterior vitreous detachment (PVD), suggesting that changes in the biochemical composition of ILM is a prerequisite to PVD.

Transmission electron microscopy of primate ILM demonstrated the presence of carbohydrate rich, Alcian blue staining, non-collagenous material that surrounded cortical vitreous and may have a role in fibrillar attachment to the lamina rara externa of the ILM.(Matsumoto *et al.* 1984)Further histochemical studies have demonstrated specific binding of lectin compounds in neural retinal, as well as an age specific high affinity binding in the ILM of young eyes which was absent in older eyes, perhaps accounting for the decreased vitreo-retinal adhesion seen in older eyes. (Russell *et al.* 1991) Figure 1.4 highlights the structure of the vitreo-retinal interface outside the vitreous base.

1.4.2 Degenerative remodeling

A spectrum of changes has been observed in the ILM of normal eyes which have been termed 'degenerative remodelling.' Foos initially described changes at the vitreous base, equator and peri-papillary region of the ILM. These changes include discontinuity of the ILM, cleft formation and separation of Muller cells. In more severe instances, vitreous strands may adhere to these degenerative clefts or to the cell membrane of Muller cells devoid of basal lamina. Further studies have added evidence to these findings, particularly in the equatorial region, where collagen fibrils may adhere to widened intercellular spaces between Muller cells and morphological reports of "tubuli" and "spiculae" arising from the ILM and inserting into the vitreous cortex have added support to this concept.(Dunker *et al.* 1997) Similarly, evidence for a dynamic vitreous base has been demonstrated by Wang J. et al where age-dependant intra-retinal synthesis of collagen was shown to penetrate the ILM and splice with cortical vitreous expanding the width of the vitreous base.(Wang *et al.* 2003) More recently, intra-retinal collagen type II packages have been found in the pre-equatorial region associated with Muller cells, and this is thought to be the result of a process of interactive remodelling where breakdown and synthesis of vitreous and ILM collagens takes place, a process similar to that which occurs in other extra-cellular matrix components subject to mechanical stress (eg. Bone and cartilage). It thought that the development of these regions of abnormal adhesion may predispose to retinal tear formation during PVD.(Green & Sebag 2006; Ponsioen *et al.* 2005; Malecaze *et al.* 1985)

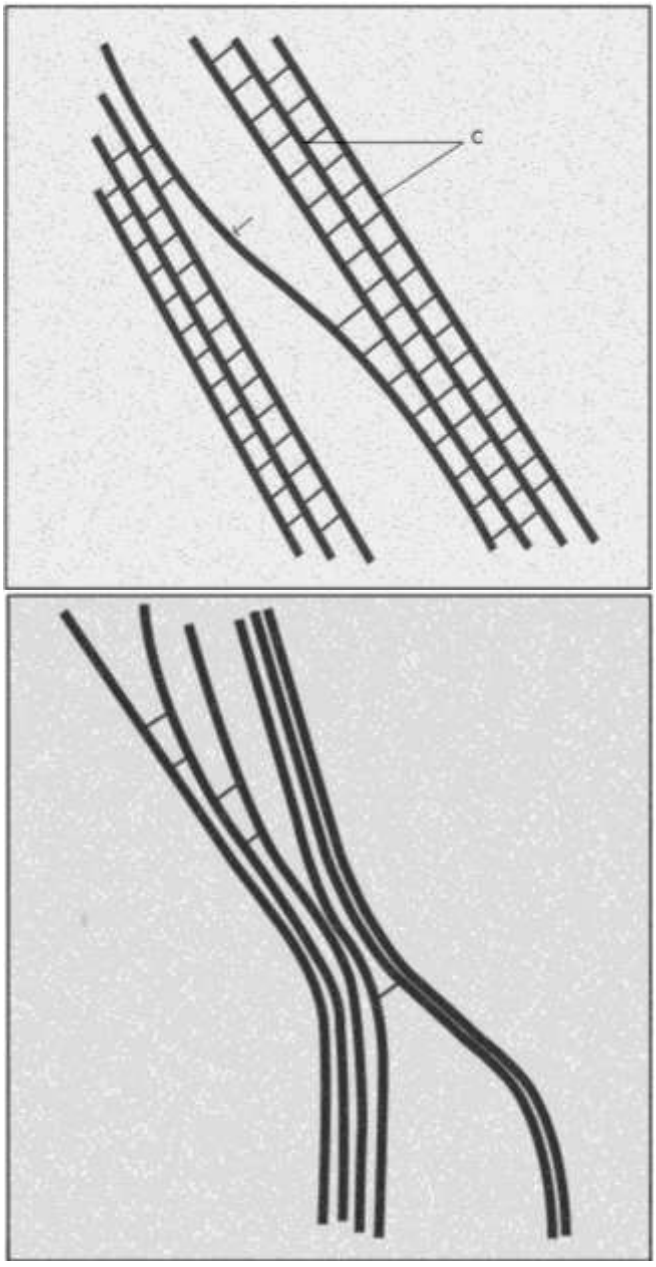


Figure 1.3ab – Figure 1.3a(above)- Illustrates the collagen (C) structure in youthful vitreous, where parallel bundles of collagen fibrils are regularly spaced apart (by chondroitin sulphate side chains – thin black lines) in a hydrophilic glycosaminoglycan matrix composed primarily of hyaluronan (grey background) which 'inflates' the collagen structure. The collagen bundles form an interconnecting network by branching between bundles. With age (figure 1.3b -below), there is loss of the regular collagen spacing and aggregation of collagen fibrils, which results in visually perceivable aggregates of fibrillar collagen.

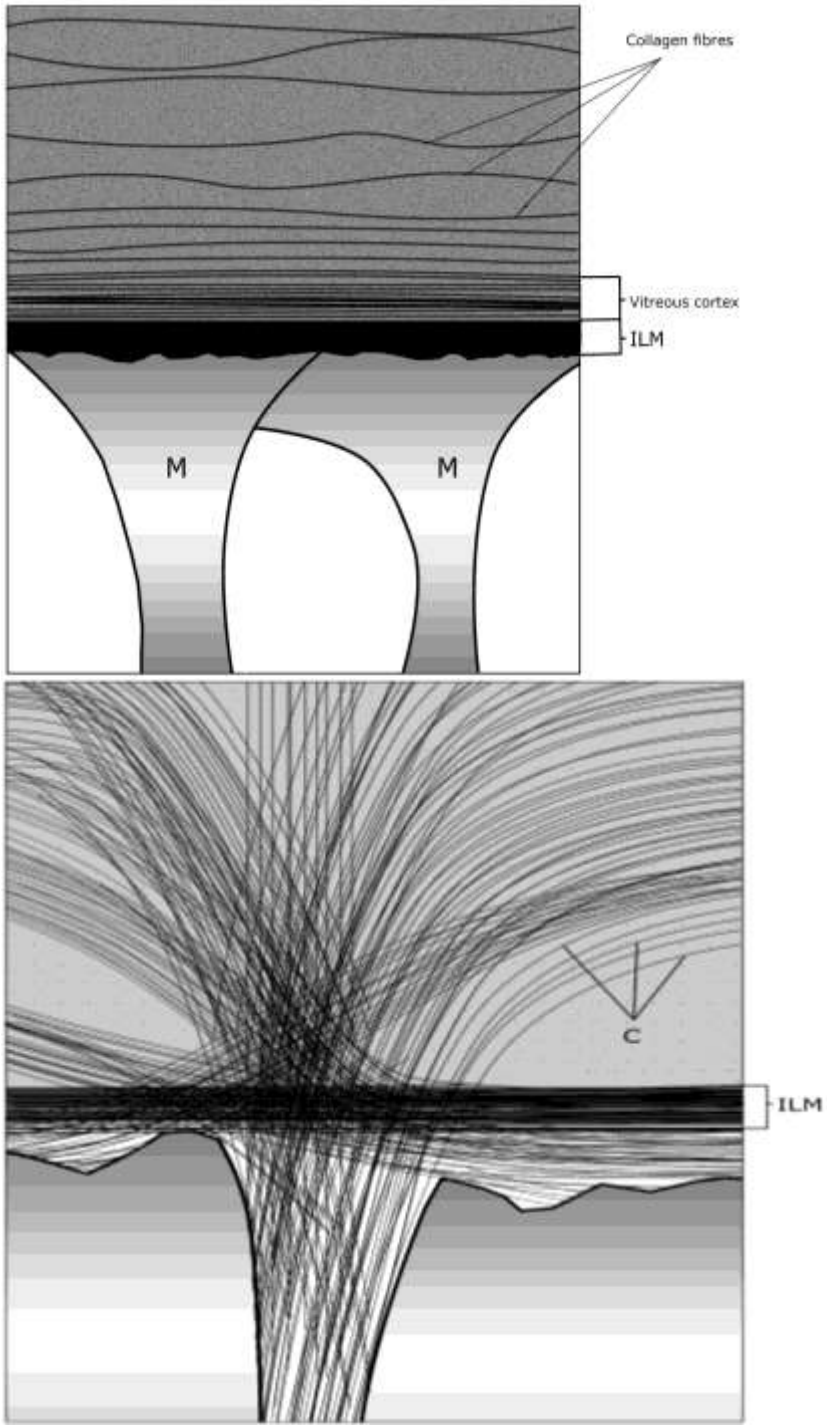


Figure 1.4ab – Schematic representation of the vitreo-retinal interface(M=Muller cell). Outside the vitreous base (Figure 1.4a-above), the dense collagen fibrils of the vitreous cortex are orientated parallel to the retina and are thought to be opposed to the retina by a molecular 'glue'. In contrast, at the vitreous base (Figure 1.4b-below), the very dense collagen fibres of the vitreous cortex are orientated radial or perpendicular to the ILM (inner limiting membrane) of the retina and insert directly into the lamina rara externa of the ILM and the ciliary epithelial cells forming an unbreakable adhesion.

1.5 Ageing changes in the vitreous

Human vitreous undergoes an age-dependant process of liquefaction. Balazs and Denlinger demonstrated that by the age of 14-18 years, 20% of the total vitreous volume is liquid. After the age of 40 years, there is a steady increase in liquid vitreous and a simultaneous decrease in gel volume. By 80-90 years, more than half of the vitreous is liquid.(Balazs & Denlinger J.L. 1982) Myopia is associated with more rapidly progressive vitreous liquefaction.(GOLDMANN 1964; Foos & Wheeler 1982; Akiba 1993) During this process, the collagen fibrils remain as the bulk component of the gel vitreous and the hyaluron concentration does not change.(Sebag & Balazs 1989) Eisners initial studies and several others since then have demonstrated that liquefaction begins in the central vitreous with the appearance of macroscopic fibres in the central vitreous. (Sebag & Balazs 1989; Eisner 1975b; Eisner 1975a; Sebag 1987) Low power microscopic studies have demonstrated that vitreous liquefaction initially occurs in pockets (lacunae) which then coalesce. (Sebag 1987; Foos & Wheeler 1982)EM studies have confirmed that the macroscopic fibres seen in the vitreous during this process are bundles of aggregated collagen fibrils which are thought to result from the breakdown of the HA/collagen macromolecular structure of the vitreous.(Sebag & Balazs 1989)

1.5.1 Pathogenesis of vitreous liquefaction

The youthful homogenous vitreous consists of bundles of collagen fibrils dispersed throughout the gel, with individual collagen fibrils spaced apart. Two progressive changes occur in the ageing vitreous: synchysis – progressive increase in liquefied spaces and syneresis – an increase in

optically dense structures.(Foos & Wheeler 1982; Balazs & Denlinger J.L. 1982; Foos 1972a; Oksala 1978) Several mechanisms are thought to be contributory to the progressive liquefaction and aggregation of collagen fibrils observed in the aging eye. Ultrastructural studies suggest there is progressive lateral fusion of collagen fibrils, resulting in visually perceivable aggregates. The fusion of these fibrils results in a redistribution of the collagen fibrils, with an increased concentration in the residual gel but a decreased concentration of fibrils in other areas, resulting in liquefaction. (Sebag & Balazs 1989) Further TEM evidence suggests a reduction in the quantity of collagen and possible enzymatic (in the form of matrix metalloproteinases) fragmentation of the collagen fibrils is important in the process of liquefaction.(Los *et al.* 2003) Bishop et al showed that progressive age-related changes on the surface of collagen fibrils may play an important role in the formation of these aggreagates.(Bishop *et al.* 2004) They found an age-dependant decrease in type IX collagen, which by virtue of its chondroitin sulphate side chains may have a role in shielding type II collagen from exposure on the fibril surface. This age dependant decline in type IX collagen and increased surface exposure of type II collagen was thought to predispose to fusion of collagen fibrils on contact. Structural age-related changes in type II collagen with an increase in molecular weight and size of the alpha-chain have been found in bovine and human eyes.(Akiba *et al.* 1993) Similarly, light-induced free-radical damage to the vitreous has caused insolubilization of the vitreous and degradation of HA which may contribute to liquefaction.(Akiba *et al.* 1994) The changes underlying vitreous liquefaction are complex, involving age-dependant structural changes in the vitreous components and the loss of normal macromolecular organisation of the gel.

1.5.2 Age related changes at the vitreo-retinal interface

In young eyes, there is an extensive and strong adhesion between the posterior vitreous cortex and the ILM of the retina. With age, this adhesion weakens and this is likely due to biochemical changes at the vitreo-retinal interface.(Sebag 1991) At birth, the vitreous base is at the ora serrata. An age dependant increase in the width of the vitreous base has been demonstrated in aged human vitreous.(TENG & CHI 1957; Wang *et al.* 2003) This increased migration of the posterior border of the vitreous base (up to 3.7mm) is more prominent on the nasal aspect and is significantly higher in males. Wang et al suggest that the extension of the vitreous base into the peripheral retina is due to the synthesis of 'new' vitreous collagen by retinal cells. Some of this newly synthesised collagen breaks through defects in the ILM and intertwines with pre-existing cortical vitreous, creating new adhesions and extending the vitreous base posteriorly. This has important implications for the pathogenesis of peripheral retinal breaks and sites of increased vitreo-retinal traction during PVD. (Wang *et al.* 2003)Recently, advanced glycation end products have been demonstrated in the aged vitreous and they may have a role in the structural alteration of the vitreous gel and the predisposition to PVD.(van *et al.* 2009)

There exists an age-dependant thickening in the ILM at the posterior pole.(Kohno *et al.* 1987) This thickening may affect the ability of Muller cells to maintain the components of the extra-cellular matrix, thus weakening vitreo-retinal adhesion.(Sebag 1991) Similarly, the coating of macromolecules may allow collagen fibrils in the cortical vitreous to stick to the ILM. Changes in the structural components of the aged vitreous may have a role in weakening peripheral vitreoretinal adhesion.(Bishop 2000)

1.6 Neurosensory retina and retinal pigment epithelium

The loss of structural interaction between the neurosensory retina (NSR) and underlying RPE causes visual loss in patients with RRD. The mechanisms of interaction between the neural retina and the RPE are complex and involve mechanical and metabolic factors.

The RPE is a polarized monolayer of epithelial cells resting on Bruchs membrame between the choriocapillaris and the NSR. The apical surface of the RPE has a complex anatomical relationship with the outer segments of the photoreceptors.(Campochiaro *et al.* 1986) The RPE microvilli extend and interdigitate the photoreceptor outer segments, they play a crucial role in disc pahgocytosis and renewal but their role in adhesion is uncertain.(Fisher S.K. & Lewis G.P. 2006) They may provide a frictional resistance or an electrostatic force that opposes separation but the magnitude of this is unknown.(Marmor M.F. 2006)

The interphotoreceptor matrix (IPM) is a viscous material composed of glycoproteins, proteoglygans and GAGs.(Adler & Klucznik 1982; Porrello & LaVail 1986) The IPM has component structures that play a role in adhesion. Cones and rods are surrounded by a specialized matrix that binds peanut and wheatgerm agglutin respectively. This matrix remains attached to both RPE and cones and stretches markedly to maintain this attachment when the NSR is peeled from the RPE, suggesting a strong bond. The evidence for this is strongest with respect to cones but is exists for rod photoreceptors also.(Hollyfield *et al.* 1989; Hageman & Johnson 1986; Johnson *et al.* 1986; Hageman *et al.* 1995) Other evidence suggesting the importance of the IPM in adhesion is gleaned from experimental studies where enzymes degrading proteoglycans have been given intra-vitreally or directly into the IPM and

have resulted in marked loss of adhesion in primate eyes.(Yao *et al.* 1990; Yao *et al.* 1994; Marmor *et al.* 1994)

Although the mechanical adhesion may largely be maintained through the IPM, post mortem and primate studies have also demonstrated the importance of active metabolism and continuous oxygenation in maintaining the adhesive strength.(Marmor *et al.* 1994; Yao *et al.* 1994; Marmor & Yao 1995; Kim *et al.* 1993)

The RPE actively transports water from the subretinal space to the choroid. This active transport, as well as dehydrating the sub-retinal space, is an important factor in maintaining adhesion. Drugs interfering with this active transport have shown to reduce retinal adhesiveness.(Marmor & Maack 1982; Marmor & Negi 1986; Hughes *et al.* 1984)Intra-ocular pressure and high retinal flow resistance through the impermeable RPE exert an outward hydrostatic force applying continual pressure on the ocular layers.(Toris *et al.* 1990; Marmor M.F. 2006)Osmotic pressure also plays a role in maintaining retinal adhesion. This is supported by in vivo studies where intravenous injections of Mannitol have increased retinal adhesion by 50%.(Kita & Marmor 1991)The combination of these factors maintain retinal adhesion under normal conditions. Once RRD occurs, these mechanisms of normal adhesion become compromised causing loss of adhesion with consequential visual loss.

1.7 Posterior vitreous detachment (PVD)

Foos originally noted the relationship between vitreous liquefaction and PVD. He noted a strong correlation between the extent of vitreous syneresis and the prevalence of PVD, showing that only a certain amount of liquefaction can be tolerated until the residual vitreous gel collapses causing

a PVD.(Foos & Wheeler 1982) PVD results from weakening of the vitreous cortex and ILM adhesion in conjunction with progressive liquefaction so that liquefied vitreous passes through the vitreous cortex into the 'retro-hyaloid' space dissecting the vitreous body from the ILM. The PVD progresses to detach anteriorly as far as the vitreous body, where strong attachments prevent further progression.(Michels RG *et al.* 1990) In order for a PVD to occur without clinical complications, the extent of liquefaction and weakening of vitreo-retinal adhesion must occur concurrently and to a similar extent.(Sebag 2004) Sebag describes a unifying concept of an anomalous PVD, where the extent of liquefaction exceeds the degree of weakening vitreo-retinal adhesion resulting in traction at this interface responsible for clinical complications such as retinal tears and RRD. In this framework, an incomplete PVD with sites of persistent vitreoretinal adhesion are need to precipitate a retinal break.(Sebag 2004)

1.7.1 Incidence of PVD

The incidence of PVD in normal populations is age dependant and varies with the method employed in examining the vitreous. Biomicroscopic examination reveals an overall incidence of 28% increasing to 53% in people of 50 years.(PISCHEL 1952)Autopsy studies demonstrated the presence of PVD in one eye in 27% of people aged 60-69 years and in 63% of people over 70 years.(Foos & Wheeler 1982) More recent ultrasonography studies support the higher frequency of PVD in the elderly, but found an overall lower incidence where 46% had a complete PVD in the 80-89 year age group.(Weber-Krause & Eckardt 1997)The incidence of PVD is higher after certain surgical procedures. One year after uncomplicated phacoemulsification only 41.4% of eyes did not have a PVD.(Mirshahi *et al.* 2009) It has also been noted that PVD occurs earlier in life and more

frequently in females than in males.(Foos & Wheeler 1982; Yonemoto *et al.* 1996)

1.7.2 Relationship between PVD and RRD

A posterior vitreous detachment is the most common cause of a retinal tear, which is the critical event in RRD formation.(Coffee *et al.* 2007) The reported incidence of retinal tears in patients with acute symptomatic PVD varies between 8-46%.(Tasman 1968; Murakami *et al.* 1983; Novak & Welch 1984; Byer 1994) A recent meta-analysis indicating that the rate of retinal break after symptomatic PVD is 21.7%.(Coffee *et al.* 2007) Discovery of retinal tears is important in preventing retinal detachment. Risk factors for developing a retinal tear with a symptomatic PVD include myopia, trauma, previous cataract surgery, lattice degeneration, previous retinal detachment in the fellow and a family history of retinal detachment.(Dayan *et al.* 1996; Boldrey 1994; Tielsch *et al.* 1996) Approximately one half of eyes with a symptomatic retinal tear and persistent vitreoretinal traction will go on to develop RRD.(Davis 1974; Shea *et al.* 1974) In contrast, asymptomatic retinal breaks in phakic eyes show a very low progression to clinical retinal detachment (<1%).(Byer 2001; Byer 1998)

1.7.3 Relationship between PVD and myopia

PVD and RRD in myopes occurs more frequently and at a younger age.(Akiba 1993) Development of vitreous liquefaction is more extensive and occurs earlier in these eyes and is significantly correlated with an axial length of >30mm.(Morita *et al.* 1995)Several studies have addressed the relationship between vitreous liquefaction and high myopia demonstrating an earlier onset of rheologic changes in the vitreous with large lacunae

formation and extensive syneresis.(Green & Sebag 2006; Morita *et al.* 1995; Grossniklaus & Green 1992)

1.8 Rhegmatogenous retinal detachment

The invention of the Ophthalmoscope by Hermann von Helmholtz in 1850 enabled physicians to visualise the retina. Further developments of the ophthalmoscope led to the discovery of retinal breaks by Albrecht von Graefes in the latter part of the 19th century. However, their association with rhegmatogenous retinal detachment (RRD) remained unknown for many years. So pessimistic was the morale at the time that in 1904, at the International Congress in Paris, retinal detachment was declared untreatable. Motivated by this declaration, a Swiss Ophthalmologist, Jules Gonin performed extensive studies into the pathogenesis of RRD and came to the conclusion that RRD is caused by small breaks in the peripheral retina. He is credited with performing the first intervention to close these retinal breaks and treat RRD - a principal which is still in use in surgical procedures today.(Sodhi *et al.* 2008; Gonin J. 1930; Wolfensberger 2003)

It has been estimated that one in 300 people will develop a rhegmatogenous retinal detachment in their lifetime.(Haimann *et al.* 1982b) In west Scotland, Ireland and southwest England up to 2% of blind and partial sight registrations are due to RRD.(Bamashmus MA. *et al.* 2004; Grey RH *et al.* 1989; Munier A *et al.* 1998)At present treatment modalities for RRD are predominantly in-patient surgical procedures. In-patients admitted with RRD may comprise up to one in five ophthalmology in-patients in specialised centres.(Li 2003)

1.9 Pathogenesis of rhegmatogenous retinal detachment

Rhegmatogenous retinal detachment (RRD) is characterized by the presence of a full thickness retinal break. This break is held open by vitreoretinal traction that allows accumulation of liquefied vitreous under the retina separating it from the RPE. Therefore, the precursors to RRD are liquefied vitreous, tractional forces that can produce and maintain a retinal break, and a break through which fluid gains access to the subretinal space. (Ghazi & Green 2002; Wolfensberger 2003)

Most eyes with retinal breaks do not develop RRD because the physiological forces attaching the NSR to the RPE are sufficient to hold the retina in place. However, when the opposing forces involved in RRD pathogenesis (vitreo-retinal traction, currents within the vitreous, gravitational forces) overwhelm the forces of attachment and liquefied vitreous fluid passes into the sub-retinal space at a faster rate than can be removed by the RPE, RRD results.(Michels RG *et al.* 1990)

A retinal break occurs when the vitreous detaches posteriorly and reaches a point of firmer attachment of the vitreous to the retina causing traction at that point. This commonly occurs around areas of exaggerated vitreo-retinal adhesion, such as the posterior margin of the vitreous base, areas of lattice degeneration or other vitreoretinal degenerations and around equatorial blood vessels. As the process of vitreous detachment continues and the vitreous remains attached to the flap of the tear, a horse-shoe tear (HST) will result. On the other hand, if the vitreous traction is strong enough to cause avulsion of the retinal tear at its base, a round hole (RH) results and the traction resolves. These are the two most common type of breaks causing RRD.(Michels RG *et al.* 1990; Ghazi & Green 2002; Machemer 1984)

An RRD is typically defined as a full thickness break in the retina that allows liquefied vitreous to enter the subretinal space and extend for 2 disc diameters.(Schepens 1951) Depending on the location of this pathology RRD can be equatorial, oral or macular. Equatorial RRD is the most common, occurring with myopia, horse-shoe tears, round holes and lattice degeneration. Oral RRD is seen in dialysis, giant retinal tears and trauma. Macular RRD is seen in high myopia and traumatic macular holes.(Ghazi & Green 2002)

The incidence of retinal tears in clinical series has been noted between 1-7.8%.(Halpern 1966; Byer 1967; Rutnin & Schepens 1967; Smith & Ganley 1972) In post-mortem studies, this has varied between 3.3-27%.(Foos *et al.* 1983; Foos 1975; Barishak & Stein 1972; Foos 1974) The incidence of retinal tears is much higher than RRD and Byer noted a prevalence of RRD and retinal breaks in a ratio of 83 to 1, with long-term studies of asymptomatic retinal breaks rarely progressing to symptomatic retinal detachment.(Byer 1967; Byer 1982; Byer 1974b)

As well as a retinal break, sustained traction and movement of liquefied vitreous are necessary for the development of RRD. Gravitational forces on superior retinal breaks can cause sustained traction and increase the progression to RRD. Rotary eye movements and shifting of the vitreous gel cause flow currents to develop in the liquid vitreous. Recent biomechanical studies demonstrate that even slight rotary eye movements can cause significant flow currents to develop in the liquid vitreous.(Repetto *et al.* 2005) When the eye rotates, the inertia of the detached vitreous causes it to lag behind the rotation of the eye and retina, and exerts a tractional force perpendicular to the site of vitreoretinal adhesion, which can cause or extend a retinal break. The sustained traction and currents in the vitreous allow the passage of liquefied vitreous through the retinal break,

dissecting the NSR away from the RPE and extending the area of detachment.(TENG & CHI 1957; Rosengren & Osterlin 1976; Michels RG *et al.* 1990)

IDIOPATHIC RETINAL DIALYSIS

Idiopathic retinal dialyses are known to account for 1.8% of RRD in large series.(Kinyoun J.L & Knobloch W.H 1984) They are defined as circumferential tears along the ora serrata with vitreous attachment to their posterior margin. They are often found inferotemporally and in a high proportion bilaterally.(Hagler 1980) Some authors suggest a developmental abnormality in their pathogenesis; others believe almost all are due to an unremembered trauma.(Verdaguer *et al.* 1975; Ross 1991) Retinal dialyses are slowly progressive and often show signs of chronicity such as demarcation lines or intraretinal cysts at presentation.(Michels RG *et al.* 1990; Kanski J & Menon J 2003)

GIANT RETINAL TEARS (GRT)

Giant retinal tears are responsible for up to 2% of RRD in large series.(Ashrafzadeh *et al.* 1973) Most tears are idiopathic.(Ang *et al.* 2010) GRTs occur more commonly in males, are associated with myopia and with certain collagen disorders, primarily Stickler syndrome. GRT is may be due to blunt trauma and in these cases there is usually avulsion of the vitreous base throughout the length of the tear.(Freeman *et al.* 1974) Giant retinal tears are defined as circumferential retinal breaks of 90 degrees or more. The tear is usually along the posterior margin of the vitreous base and rarely they may occur at the anterior margin in the ciliary epithelium. As a direct result of circumferential extension, GRTs tend to adopt a rolled or inverted

edge and can require complex surgical repair.(Michels RG *et al.* 1990; Kanski 1975)

MACULAR HOLE (MH)

Macular holes are an uncommon cause of RRD. In the West their incidence is estimated between 0.5 – 1% being higher in Asian countries. Macular hole RRD occurs more frequently after blunt trauma or in highly myopic eyes.(Zhang & Hu 1982; Leaver & Clearly 1975)

1.10 Clinical Features of rhegmatogenous retinal detachment

SYMPTOMS

Symptoms of RRD are not reliable indicators of RRD. Most patients with RRD notice a variety and often a combination of symptoms including floaters (60%) or flashes (55%) often followed by a shadow and loss of vision in up to 80% of cases. Over 75% of patients may present with sudden painless loss of vision. Other symptoms include dyschromatopsia, ocular pain and diplopia. A small proportion (7%) of patients may be asymptomatic.(Polkinghorne & Craig 2004a; Kanski J & Menon J 2003)

SIGNS

An acute RRD typically has an opaque, convex configuration and undulates freely with eye movements with a loss of underlying choroidal pattern. There may be pigmented cells in the anterior vitreous (Schafer's sign), vitreous hemorrhage and PVD (seen as a Weiss ring on biomicroscopy). The intra-ocular pressure is usually lower in the affected eye and if extensive, there may be a relative afferent papillary defect. Long-standing or chronic RRD may show retinal thinning, the presence of intra-retinal cysts (if present for

over 1 year) or subretinal demarcation lines. These subretinal demarcation lines are caused by the proliferation of RPE cells at the junction of the attached an unattached retina, and my take several months to develop.(Kunimoto DY *et al.* 2004; Kanski J & Menon J 2003)

PROLIFERATIVE VITREORETINOPATHY (PVR)

PVR is an anomalous scarring condition caused by cellular proliferation and contraction of membranes on the posterior surface of the detached hyaloid membrane, the inner retinal membrane (epiretinal membrane) and the outer retinal membrane (subretinal membrane). This membranous proliferation causes tractional bands within the vitreous, retinal folds (star folds) and increased retinal rigidity. It the commonest cause of surgical failure in RRD and can complicate surgery if present preoperatively. It is thought to be present in approximately 7% of RRD cases and is much more likely if several features are also present: GRT, multiple retinal tears, uveitis, aphakia or vitreous hemorrhage. The classification of PVR is defined by the American Academy of Ophthalmology and varies in severity from Grade A (minimal) to Grade D (massive).(Pastor 1998; Girard *et al.* 1994; 1983)

1.10.1 Treatment of rhegmatogenous retinal detachment

The management of asymptomatic RRD is controversial, with studies supporting conservative (observation) management and surgical treatment in high-risk cases or cases with likely progression to involve the macula. Symptomatic RRD is an indication for surgical repair.

PROPHYLAXIS

No defined guidelines exist for preventative treatment of RRD in patients with predisposing retinal degenerations. Recent evidence supports

prophylactic treatment for symptomatic retinal tears, retinal breaks threatening progression, high risk fellow eyes of non traumatic GRT and retinal breaks present before cataract surgery.(Kazahaya 1995; Ang *et al.* 2009)

SURGICAL APPROACHES

Scleral buckling

Scleral buckling surgery involves an inward indentation of the sclera using a 'buckle' explant. These may be radial, partially circumferential or encircling. The principal is to close the retinal break by apposing the RPE to the neurosensory retina and to reduce dynamic vitreoretinal traction at the site of the break. It is the method of choice for uncomplicated RRD, has proven long-term effectiveness in retinal attachment and current evidence suggests a final anatomic success rate of 94%.(Schwartz *et al.* 2002; Kanski J & Menon J 2003; Sodhi *et al.* 2008)

Pars Plana Vitrectomy (PPV)

Initially used in complicated cases of RRD pars plana vitrectomy is becoming an increasingly performed procedure for all cases of RRD. PPV is a microsurgical procedure which aims to remove the posterior hyaloid face, relieve vitreoretinal traction, manipulate and reattach the retina and create space for internal tamponade. Numerous agents can be used for internal tamponade including gas (sulphur hexafluoride, perfluoropropane), heavy liquid (perfluorocarbon) and silicone oil. The instrumentation and techniques used for PPV are continually being advanced and improved. PPV holds numerous advantages over scleral buckling including better visualisation of the posterior pole as well as the ability to remove and manipulate opacities and proliferative membranes. PPV has a high final success rate of up to 95%

in RRD complicated by PVR, pseudophakia or multiple breaks.(Sodhi *et al.* 2008; Kanski J & Menon J 2003; Schmidt *et al.* 2003)

Pneumatic retinopexy

This technique was initially described in 1986. It involves the injection of an intra-vitreal gas tamponade without PPV or scleral buckling. It is useful in selected cases; uncomplicated RRD caused by small closely clustered retinal breaks in a narrow area of the superior retina. It has the advantage of being minimally invasive with reduced recovery time, however because it does not relieve vitreo-retinal traction, a re-detachment risk persists. Primary success rate is 64%, and final success rate is 91%. (Hilton & Grizzard 1986; Sodhi *et al.* 2008; Kanski J & Menon J 2003)

1.10.2 Visual outcome after RRD surgery

Macular attachment at presentation is one of the most important predictors of final visual outcome. Despite advances in surgical treatment functional results after RRD repair remain poor with visual acuity of >20/40 (6/12) only achieved in 28.4% of cases where the macula is detached.(Pastor *et al.* 2008)This finding is further supported by Minihan et al, who demonstrated that both primary and final anatomic success rates were similar in two studies conducted 20 years apart (80-84% in 1979 and 89-94% in 1999).(Minihan M. *et al.* 2001) There has been much controversy over which surgical method should be used to treat RRD.(Sodhi *et al.* 2008) However there has only been one large randomized multi-centre trial which demonstrated no difference in final visual acuity between PPV and scleral buckling in pseudophakic cases but an improved final visual outcome with scleral buckling in phakic eyes.(Heimann *et al.* 2007)

1.11 Risk factors influencing RRD development

1.11.1 Myopia

Myopia is an error of refraction primarily caused by increased axial length but may also be caused by corneal or lenticular changes. Myopia and especially high myopia have been established as one of the major risk factors for RRD.(C.L.Schepens 1961; Cambiaggi 1964; Druault-Toufesco S 1922) High myopia is defined as a spherical equivalent of -6.0 dioptres (D) or an axial length (AL) of at least 26mm.(Curtin BJ 1985) Myopic eyes are more likely to develop retinal tears after PVD than non-myopic eyes.(Grand 2003; Byer N.E 1979) It has been reported that an eye with a spherical equivalent refractive error of between -1 to -3 D has a fourfold increased lifetime risk of RRD compared to a non myopic eye, increasing to tenfold with a refractive error of >-3D. (Austin *et al.* 1990; The Eye Disease Case-Control Study Group 1993) Furthermore, an increase in axial length of 1mm increases the risk of RRD by a hazard ratio of 1.3.(Ninn-Pedersen & Bauer 1996) Myopic patients are more likely to have RRD at a younger age and more likely to develop bilateral RRD. (Burton *et al.* 1983; Michels RG *et al.* 1990; The Eye Disease Case-Control Study Group 1993; Burton 1989; Li 2003; Yonemoto *et al.* 1994)

Myopia predisposes to RRD through increased vitreous liquefaction, PVD at a younger age and an increased incidence of predisposing vitreoretinal degenerations such as lattice degeneration. The presence of specific factors and biochemical changes which weaken vitreo-retinal adhesion in myopic eyes remains to be resolved. It has been postulated that compromised Muller cell function may play a role in weakening adhesion as is

demonstrated by abnormal b-wave amplitude in high myopes on electroretinogram.(Sebag 1991; Morita *et al.* 1995) Age related changes such as ILM thickening cannot account for the early onset of RRD noted in these patients. Developmental studies where myopia was induced in newly born chicks demonstrated an increase in the volume of vitreous in myopic compared to non-myopic eyes, indicating that perhaps a developmental factor may influence early liquefaction in highly myopic eyes.(Pickett-Seltner *et al.* 1992) In humans, this is supported by connective tissue disorders such as Stickler syndrome and Wagner syndrome exhibiting vitreous degeneration and varying degrees of myopia, suggesting that a type of developmental dysgenesis of the vitreous in myopic eyes may have an important role in conferring this increased risk.

1.11.2 Trauma

Blunt trauma is an important cause of retinal breaks and subsequent RRD. A blunt contusion is thought to cause a change in the in the antero-posterior diameter of the eye followed by a sudden compensatory lengthening. This causes marked vitreo-retinal traction and can result in retinal breaks such as peripheral dialysis, avulsion of the vitreous base, macular holes and horse-shoe shaped tears at the posterior margin of the vitreous base or equator. These breaks usually occur at the time of impact and can progress slowly.(Ghazi & Green 2002; Sodhi *et al.* 2008; Cox *et al.* 1966)Penetrating ocular injury may directly cause RRD and these tend to have a poor prognosis.(Cox & Freeman 1978)

1.11.3 Cataract surgery

Uncomplicated cataract extraction regardless of method employed increases the risk of retinal detachment. This is thought to be due to a higher rate of PVD after cataract extraction as a result of more tractional forces after lens

removal and a lower concentration of hyauronic acid contributing to vitreous collapse.(Ghazi & Green 2002) The long-term incidence of retinal detachment after cataract extraction varies with the method of cataract surgery - studies have estimated this at 0.4-3.6% for intra-capsular cataract extraction, 0.55-1.65% for extra-capsular cataract extraction and 0.41%- 1.65% for phacoemulsification. (Lois & Wong 2003; Boberg-Ans *et al.* 2003) The incidence is much higher if cataract surgery is complicated by posterior lens capsule rupture (up to 7%). Up to half of RRDs after cataract extraction will occur within the first year after the initial surgery. The cumulative risk of RRD post cataract surgery increases linearly with time. (Javitt *et al.* 1994; Norregaard *et al.* 1996)The cumulated six year risk of RRD is increased by a factor of 6 to 8 after cataract surgery and this risk continues to increase for at least eight years after surgery, possibly up to 20 years postoperatively. (Norregaard *et al.* 1996)'(Erie *et al.* 2006) (Boberg-Ans *et al.* 2006)

1.11.4 Posterior capsulotomy

Posterior capsule opacification occurs in 20-30% of eyes after cataract surgery and is the most common post-cataract surgery complication. The treatment of choice for this is Nd:YAG capsulotomy (Nd:YAG). The incidence of RRD after Nd:YAG is reported between 0-4.1% after a four year follow up.(Lois & Wong 2003) Few studies have estimated the risk of Nd:YAG as a risk factor independent of cataract surgery, however, Javitt et al found the relative risk of RRD in patients who underwent Nd:YAG compared to those who didn't was 3.9.(Javitt *et al.* 1992) It is thought that an alteration in vitreous structure, or disruption of the anterior hyaloid membrane may be responsible for this increased incidence. Changes in energy used and size of

capsule disruption did not appear to influence risk of RRD.(Dardenne *et al.* 1989; Koch *et al.* 1989; Rickman-Barger *et al.* 1989)

1.12 Peripheral retinal degeneration

There are several peripheral retinal lesions which cause anomalies in vitreo-retinal adhesion predisposing to RRD. These include lattice degeneration, oral bays, meridional folds, retinal tufts, white without pressure and pavestone degeneration.

1.12.1 Lattice degeneration

Lattice degeneration was first described by Gonin in 1930 as "brilliant white trellis" and is now known to be the most common vitreo-retinal degeneration.(Gonin J. 1930) Lattice degeneration occurs early in life and the peak prevalence is in the second decade. The prevalence of lattice degeneration is similar in worldwide reports: In the US it is seen in 6-8% of normal eyes (Byer 1965; Straatsma BR *et al.* 1974) and in Japan in 9.5%.(Shiomi Y 1981) It is frequently associated with myopia and is the most important peripheral retinal degeneration predisposing to RRD.(Lewis 2003) The prevalence of lattice increases with increasing myopia from 4.3% in emmetropia to 14% in myopia of >-3D. Its prevalence is also associated with increasing axial length being present in 15% of eyes with an axial length of at least 30mm and in less than 7% of eyes with axial lengths less than 27mm. (Burton 1989; Michels RG *et al.* 1990; Ashrafzadeh *et al.* 1973) Lattice degeneration is bilateral in up to 48% of affected cases. Lattice lesions are usually numerous with the average between 2 and 24 lesions. It is found most commonly in the vertical meridian and inferotemporal retinal quadrants.(Byer 1965; Byer N.E 1979) Lattice degeneration has numerous morphological features, but the pathogenesis remains largely unknown.

Lattice degeneration often exhibits pockets of overlying cortical vitreous liquefaction. Foos examination of eyes at autopsy demonstrated that this is not associated with the central progressive liquefaction occurring as a natural process of ageing, indicating that PVD is not the basis for the higher rate of RRD in these eyes.(Foos & Simons 1984) EM studies have demonstrated several retinal and vitreous abnormalities in regions of lattice degeneration. These include alterations in ILM and absence of basement membranes over the surfaces of lesions as well as an increased presence of astrocytes.(Straatsma BR *et al.* 1974; Streeten & Bert 1972)

Clinically, lattice degeneration is a circumferentially distributed, sharply demarcated, elliptical area of peripheral retinal thinning with variable pigmentation. These lesions are often described as lattice-like white lines or shiny snail track variations associated with ovoid pigmented craters. They are often characterised by a criss-crossing network of hyalinised vessels with exaggerated vitreoretinal attachments along the edge of the lesion.(Byer 1975; Byer N.E 1979) Tractional retinal tears associated with lattice are frequent, noted in 64-83% of clinical series.(Byer N.E 1979) The most common cause of RRD associated with lattice is due to tractional retinal tears which can occur at the lateral and posterior margin of a lattice lesion during PVD due to strong retinal adhesion. However, secondary atrophic retinal holes within areas of lattice can be present in up to 30% of eyes and can cause a localised detachment which may become progressive.

Radial perivascular lattice is a 'lattice-like lesion' predisposing to RRD. It is distinct from lattice degeneration. It is usually found post-equatorially and is characterised by broad ill-defined lesions. There is a high incidence of retinal tears associated with this lesion, and it typically occurs with Stickler syndrome(Ghazi & Green 2002; Lewis 2003; Sodhi *et al.* 2008)

1.12.2 Lattice degeneration and the risk of RRD

Although lattice degeneration is a risk factor for RRD, the large majority of patients with lattice do not develop RRD. The incidence of RRD from atrophic holes and tractional tears associated with lattice is estimated between 0.3-0.5%. Hyams and Byers followed a group of patients with lattice degeneration for up to 10 years and found that only 1.4% progressed to RRD. Progression to RRD is more likely in young myopic eyes with lattice degeneration due mainly to round holes within areas of lattice degeneration. (Byer 1974a; Byer N.E 1979; Straatsma BR *et al.* 1974; Hyams *et al.* 1974) At present there is no scientific evidence to suggest beneficial prophylactic treatment of eyes with lattice with or without associated retinal breaks and prophylaxis is considered on a case per case basis.(Lewis 2003)',(Michels RG *et al.* 1990; Byer 1974a)

1.12.3 Oral bays

Oral bays are areas of retinal indentation near the ora serrata and may be completely surrounded by retinal tissue (enclosed) or incompletely surrounded by retinal tissue (partially enclosed). Oral bays are present in 3% of eyes in a large autopsy series. Enclosed oral bays are more significant for RRD development as they mimic retinal holes. In a series of 1000 autopsy eyes, a retinal tear was associated with an oral bay in 17% of cases.(Michels RG *et al.* 1990; Spencer *et al.* 1970a)

1.12.4 Retinal tufts

Retinal tufts may be non-cystic, cystic or zonular. Non-cystic tufts are not associated with retinal detachment. Cystic tufts are congenital projections of

fibroglial tissue into the vitreous cavity and may be associated with up to 6% of retinal holes. Cystic tufts are frequently located around the equator; they are unilateral in 80% of cases and are present in 5% of the population. They represent areas of strong vitreoretinal adhesion and are associated with retinal tears. Tears around areas of cystic tufts have been reported as being responsible for up to 10% of RRD. However the longterm risk of RRD from a cystic retinal tuft is 0.28%.(Byer 1981; Lewis 2003; Murakami-Nagasako & Ohba 1982)

Zonular tufts are anterior projections of fibroglial tissue which are continuous with the lens zonlules. Zonular tufts are present at birth, bilateral in 15% and usually located in the nasal quadrant. They provide a means whereby direct zonular manipulation can cause retinal traction and rarely may be associated with RRD after cataract extraction.(Ghazi & Green 2002; Foos 1969)

Meridional folds and complexes are found in 12% and 20% of normal eyes respectively. Meridional folds are elevated radial retinal folds with the majority emerging from dentate processes. Histologically, they are areas of thickened retina with cystic degeneration and may have vitreous attachment. Meridional complexes describe the presence of a dentate process in the same meridian as a ciliary process. Through abnormal vitreous attachment, both may be associated with an increased incidence of retinal breaks, however the association with risk of RRD is weak.(Spencer *et al.* 1970b; Michels RG *et al.* 1990; Ghazi & Green 2002)

1.12.5 Degenerative retinoschisis

Retinoschisis describes a splitting of the neurosensory retina into two layers, an inner (vitreous) layer and an outer (choroidal) layer. Degenerative

retinoschisis is associated with retinal detachment in 2-6% of cases.(McPherson *et al.* 1981) First delineated by Straatsma and Foos(Straatsma & Foss 1973), there are two types of degenerative retinoschisis based on the type of pre-existing cystoid degeneration that progresses to cause retinoschisis. Degenerative retinoschisis is preceeded by peripheral cystoid degeneration of the retina which can be either typical (flat) or reticular (bullous). Typical cystoid degeneration is usually bilateral and results in cystic retinal spaces which coalesce at the ora serrata and extend circumferentially and posteriorly. The cystic spaces develop between the outer plexiform and inner nuclear layers of the retina. Typical cystoid degeneration is not associated with any pathological lesions or anatomical variations. Reticular cystoid degeneration is less common than the typical form and is frequently nasal and in the inferotemporal quadrant. Reticular cystoid degeneration is the more severe form, causing loss of ganglion cells and the development of cystic spaces bounded by the inner plexiform layer and the thin internal limiting membrane. Typical retinoschisis is present in 1% of autopsy eyes, is bilateral in 33%, associated with a hypermetropic refractive error in up to 80% and is usually located inferotemporally. Reticular retinoschisis is slightly more common (1.6%), extends posterior to the equator in nearly all cases and is also located inferotemporally.(Straatsma & Foss 1973; Michels RG *et al.* 1990)

There are 2 types of RRD associated with degenerative retinoschisis. The first is a localized, relatively stable RRD which occurs when there is a hole in the outer layer only. This is the most common form of RRD associated with retinoschisis and is found in 6.4% of clinically followed up eyes.(Byer 1968) Rarely, progressive symptomatic RRD can occur from retinoschisis and retinal breaks in both the outer and inner layers. Data on the proportion of cases that develop this complication varies significantly, but is thought to be

quite low (0.5%). Treatment for RRD associated with retinoschisis is usually limited to patients who develop symptomatic, progressive RRD.(Lewis 2003)

1.12.6 Pavingstone degeneration

Sometimes referred to as 'chorioretinal atrophy' or 'cobblestone degeneration,' pavingstone degeneration are lesions of flat, pale and well demarcated appearance in the retinal periphery anterior to the equator. First described in 1855 these lesions are seen in 35% of over 80 year olds.(Michels RG *et al.* 1990) They are bilateral in the majority of myopes (57%) and are present in over 50% of eyes with an axial length greater than 28mm. Histologically they show absence of underlying choriocapillaris and loss of RPE. The pathology of these lesions is linked to choroidal vascular insufficiency. Pavingstone degeneration does not predispose to RRD but if involved in an area of retinal detachment, a secondary break may occur which can be difficult to identify clinically.(O'MALLEY *et al.* 1965)

1.12.7 White without pressure

The terms "white with pressure" (WWP) and "white without pressure" (WWOP) refer geographic areas of white discolouration in the peripheral retina. The former occurring with sclera indentation, the latter, considered to be more important, is apparent without indentation. These areas are circumferentially orientated usually in the temporal quadrants and extend for a variable distance posteriorly with an irregular sharply defined border.(Lewis 2003) WWOP is found in 15-22% of normal eyes. It is much more common in young myopes, with reports indicating that over 30% of young individuals with an axial length of greater than 33mm have WWOP.(Pierro *et al.* 1992; Shukla & Ahuja 1982) Histologically, there is localised atrophic change, and loss of the ILM with some authors speculating

that WWOP may be an earlier stage of another degeneration.(Curtin BJ & Karlin DB 1971; Pierro *et al.* 1992) WWOP is not thought to confer a significant risk of developing RRD.

CHAPTER 2 The epidemiology of rhegmatogenous retinal detachment

2.1 Comprehensive review of RRD epidemiology

In this section I present a comprehensive review of previous population based studies examining the epidemiology of rhegmatogenous retinal detachment.

I conducted a Medline database search from January 1970 to January 2009 using search terms 'incidence,' 'population,' 'epidemiology' and 'rhegmatogenous,' 'retinal detachment' in different combinations. The search engine Google Scholar was also used to identify relevant articles published in books, journals and websites. Further articles were identified by tracking the references of relevant publications. Studies were eligible if they were observational and designed to estimate RRD incidence. Paediatric studies were excluded. Articles published in other languages were included and if needed translated. Articles published as letters or abstracts were excluded. (see Figure 2.1)

The titles and abstracts of all articles found were reviewed. I developed a checklist (Appendix A), based on the STROBE recommendations,(von *et al.* 2008) and each article was given a methodology score out of 20. A consensus on inclusion criteria was agreed with a second investigator (BWF) and studies meeting the following criteria were included:

-Recruitment period of at least 1 year

-Clear case inclusion criteria

-Clear statistical data and an effort to report 95% confidence intervals for incidence rate

-Reporting of age specific incidence rates

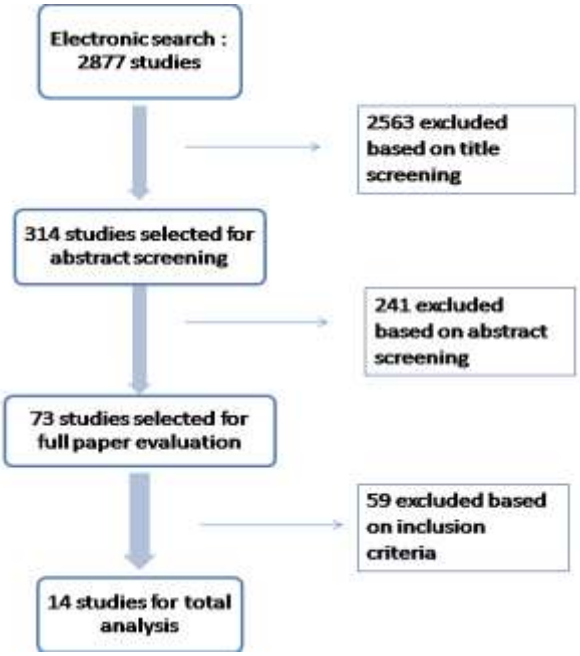


Figure 2.1 – Flowchart of literature search on population based studies of RRD.

If necessary, the 95% confidence intervals of incidence rates were calculated from demographic data assuming Poisson distribution. The age and sex-specific incidence and geographic distribution of incidence estimates were examined.

Selected studies

A total of 14 studies were included (see Figure 2.1). Eight were retrospective assessments from hospital records (5) or ICD coded data (3). Four studies established prospective recruitment from treatment centres. One was a questionnaire survey and another, a local population survey. Only 2 studies

achieved the maximum methodology score. All studies principally report the incidence of RRD cases attending hospital services for diagnosis or treatment.

2.2 Epidemiology of rhegmatogenous retinal datachment

2.2.1 Incidence and geographic variation

The first comprehensive population-based estimates of RRD were conducted in Iowa and Minnesota.(Haimann *et al.* 1982a; Wilkes *et al.* 1982) The former was based on a hospital register and a questionnaire to all 24 ophthalmologists in Iowa city and surrounding regions. The latter study involved a database search for all patients presenting to hospitals in Rochester, Minnesota between 1970 and 1978. Despite a large difference in case size and methodology, both reported an overall annual incidence of approximately 12 per 100,000.

At a similar time in Europe, two studies reported differing annual incidences of 6.9 and 10.6 per 100,000 in Finland and Sweden.(Laatikainen *et al.* 1985; Tornquist *et al.* 1987a) The Finnish study was an operative series from Helsinki University Hospital between 1978 and 1981. The Swedish study included all patients admitted with RRD to a medical centre over ten years (1971-1981). From the data, it is unclear why the reported incidences vary by 40%. Excluding aphakia, the reported incidence of RRD varies between 5.4 and 8.5 per 100,000 of population.

Epidemiological analyses of RRD have also used large centralised computer databases – The Rochester Epidemiology Project identified Olmstead county residents admitted with RRD over 20 years (1976-1995), giving the highest

reported overall incidence of 17.9 per 100,000.(Rowe *et al.* 1999) One Swedish study established a complex, computer based national register and analysed over 1,000 patients with RRD reporting an overall incidence of 14 per 100,000.(Algvere *et al.* 1999) The first Asian study reviewed 1,126 Singapore residents having operative repair of RRD over four years reporting a constant annual incidence of 10.5 per 100,000. (Wong *et al.* 1999) Overall, centralised databases may provide higher case capture, however previous reports have demonstrated a large (~2-fold) variation.

Two Chinese studies (Li 2003; Zou *et al.* 2002) have estimated differing annual incidences between 7.98 and 14.4 per 100,000, with the proportion of myopia being high (between 66-68%) in both studies. In Beijing, patients were recruited bimonthly from 35 centres. The Shanghai study included hospital cases and out-patients from the local health network, showing an annual increase in incidence over the four year study period, from 11.3 to 17.9 per 100,000 of population. Similarly, a five year review of patients undergoing surgical repair showed the incidence of two regions only 8 miles apart varied between 11.3 per 100,000 (Wolverhampton) and 6.3 per 100,000(Walsall). (Mowatt *et al.* 2003)

The reported incidence, study design, case inclusion and relative sample size of population based epidemiological analyses of RRD is summarised in table 2.1 and figure 2.2, demonstrating the wide range reported. Of the prospectively conducted studies, only 2 have attempted to include out-patient cases.(Li 2003; Zou *et al.* 2002) Several studies have a small sample size introducing a potential for sampling error. Examining the eight studies with a sample size >300 cases reveals a median annual incidence rate of 10.5 (IQR 8.1 - 13.2) per 100,000 of population. However, the inclusion of trauma and re-operations differs between all reports, with 7 of 14 studies

not specifying if re-operations were included. Excluding all other cases and examining non-traumatic phakic cases only reveals a similar variation between 5.3 and 12.6 cases per 100,000 of population. (see figure 2.3)

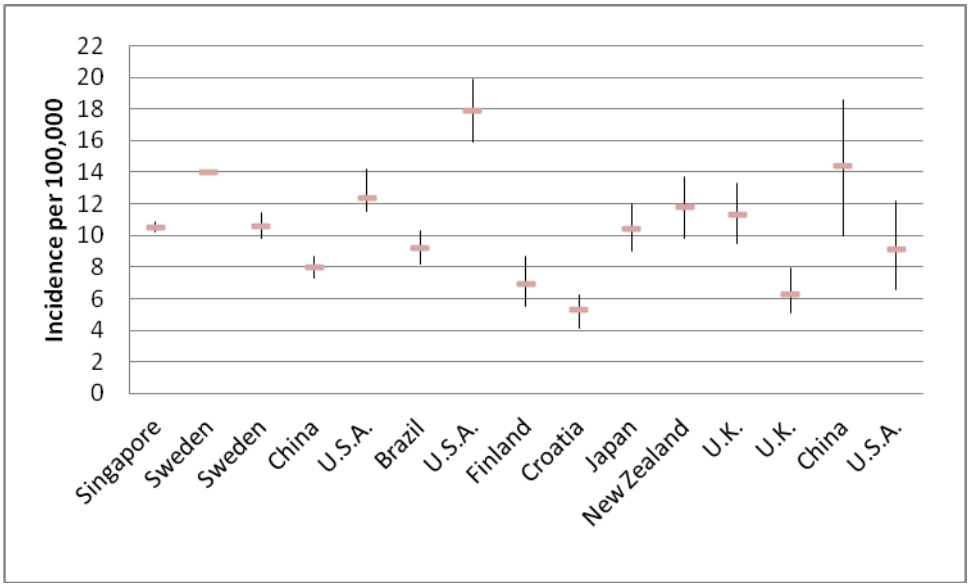


Figure 2.2 – Overall reported total incidence rates (per 100,000 of population) and associated confidence intervals of RRD in previous population based epidemiology studies ordered by sample size.

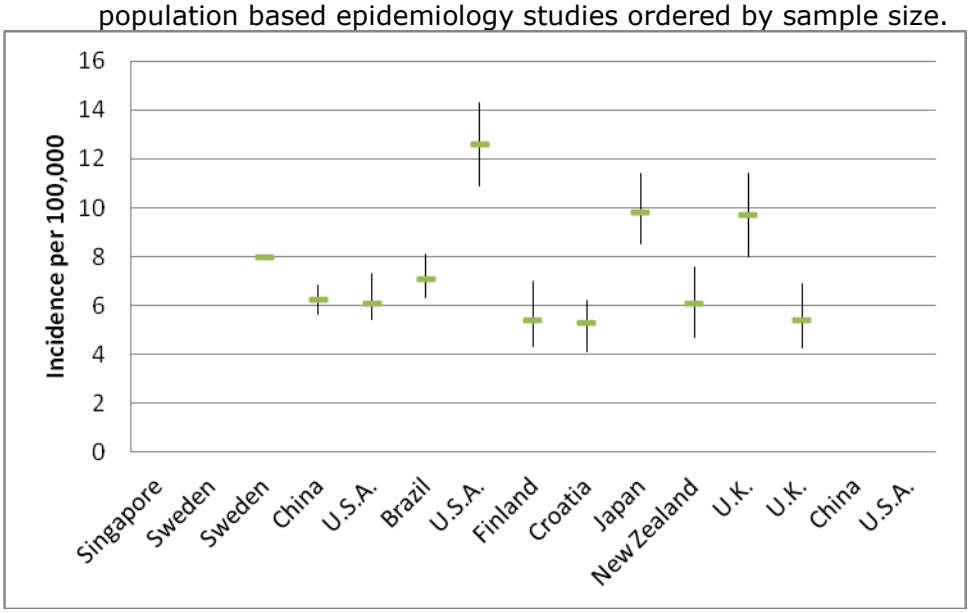


Figure 2.3 – Overall reported incidence rates and associated confidence intervals of non-traumatic phakic RRD in previous population based studies ordered by sample size.

2.2.2 Age Distribution

RRD develops from retinal breaks occurring at physiologic or pathologic sites of firm vitreo-retinal adhesion during posterior vitreous detachment (PVD).(Ghazi & Green 2002) The prevalence of PVD increases with age; occurring in 11% of 60-69 year olds, 46% of 80-89 year olds and occurring earlier in myopes. (Weber-Krause & Eckardt 1997)

The largest annual incidence rate of RRD is seen in the elderly with studies supporting a bimodal distribution and a secondary peak in younger ages (20-30 years) attributed to highly myopic patients.(Polkinghorne & Craig 2004b; Li 2003; Wong *et al.* 1999; Sasaki *et al.* 1995; Haimann *et al.* 1982a) The highest incidence rate of RRD was found in the 60-69 age group with rates varying between 19 and 27 per 100,000, (Haimann *et al.* 1982a; Sasaki *et al.* 1995; Mowatt *et al.* 2003; Zou *et al.* 2002; Li 2003) however unexplained incidence rates as high as 40 and 70 per 100,000 have been reported.(Rowe *et al.* 1999; Polkinghorne & Craig 2004b; Limeira-Soares *et al.* 2007) (Figure 2.4)The strongest association was noted between advancing age and non-traumatic phakic RRD.(Haimann *et al.* 1982a; Li 2003; Sasaki *et al.* 1995; Mowatt *et al.* 2003; Ashrafzadeh *et al.* 1973) After 60 years the risk of developing RRD can be 17-20 times greater than a cohort under 30 years.(Tornquist *et al.* 1987a) The incidence rate of RRD in under 20 year olds was the lowest noted; <3 per 100,000, similar to previous paediatric series. (Rosner *et al.* 1987; Wong *et al.* 1999; Li 2003; Limeira-Soares *et al.* 2007; Haimann *et al.* 1982a; Polkinghorne & Craig 2004b)

Despite a similar distribution of over 60 year olds in study populations, the incidence of RRD in this age group has also shown variation. (See table 2.2) In Beijing and Finland 14% and 15.2% of the population were over sixty and the incidence rates were comparable at 7.98 and 6.9 per 100,000.(Li 2003;

Laatikainen *et al.* 1985) However, in Singapore and Brazil, the proportion of the population over sixty was <10%, but the reported incidences in this group differed more than two-fold, suggesting other influencing factors.(Wong *et al.* 1999; Limeira-Soares *et al.* 2007)

2.2.3 Gender

While some reports indicate a sex distribution corresponding with that of the general population,(Haimann *et al.* 1982a)'(Laatikainen *et al.* 1985) most indicate a preponderance of males,(Mowatt *et al.* 2003; Ivanisevic *et al.* 2000; Rosman *et al.* 2001; Polkinghorne & Craig 2004b; Limeira-Soares *et al.* 2007)(Male-to-Female Ratio-1.3:1 to 2.3:1)and a minority find that females predominate in the phakic non-traumatic group. (Male-to-Female Ratio– 1:1.16 to 1:1.4) (Tornquist *et al.* 1987a; Sasaki *et al.* 1995) A large Singapore study found that males are twice as likely to require surgery. This may be related to an inherent gender risk, though an increased rate of ocular trauma was contributory. (Wong *et al.* 1999) Furthermore, in myopic populations' males tend to predominate, although with age, this gender based refractive difference may be less significant. (Schepens & Marden 1966; Hirsch & Ditmars 1969; Hirsch M.J. 1953; Bourne *et al.* 2004; Dandona *et al.* 2002; Sawada A *et al.* 2008; Attebo *et al.* 1999) Nonetheless, the higher proportion of myopia in young males may partly explain the imbalanced gender distribution in some studies.

2.2.4 Seasonality

Seasonal variation in RRD incidence with a summer peak and winter trough has been reported.(Thelen *et al.* 1997; Ghisolfi *et al.* 1986; Paavola *et al.* 1983; Laatikainen *et al.* 1985) The effect of light and temperature on the vitreous has been implicated.(Thelen *et al.* 1997) In some studies, no

seasonal variation was found,(Tornquist *et al.* 1987a; Zou *et al.* 2002; Ivanisevic *et al.* 2002) and others note a winter peak and a summer trough.(Al Samarrai 1990)

2.2.5 Ethnicity

Of the few studies performed, ethnic variation significantly affects RRD incidence. The reason for this is unknown. In Singapore, RRD incidence in Chinese is three times higher than in Indians (11.6 per 100,000 and 3.9 per 100,000) (Wong *et al.* 1999) despite minimal variability of risk factors (myopia, lattice degeneration, previous intra-ocular surgery and trauma). A UK study noted a 3-fold lower incidence in Asians compared to Caucasians.(Mowatt *et al.* 2003) Numerous reports suggest the incidence of RRD in Blacks is lower than Caucasians.(Av-Shalom *et al.* 1967; Weiss & Tasman 1978; BROWN & THOMAS 1965; Kaimbo *et al.* 1986; Peters 1995; Aboise A 1979; Yorston & Jalali 2002) However, to date there have been no population-based studies of RRD incidence in developing countries. (Yorston & Jalali 2002) Despite reported variations, post mortem studies have not demonstrated racial variation in the prevalence of predisposing ocular factors to RRD. (Foos *et al.* 1983)

2.3 Clinical associations

2.3.1 Trauma

Traumatic RRD shows a male predominance affecting younger age groups. Series in Finland, Sweden, Beijing and Iowa found between 6.3 and 12.2% of RRDs were traumatic with the majority being young males. (Tornquist *et al.* 1987a; Haimann *et al.* 1982a; Li 2003)'(Laatikainen *et al.* 1985) In New

Zealand 16.4% of RRDs were associated with ocular trauma with nearly 90% under the age of fifty.(Polkinghorne & Craig 2004b) In the majority of studies, the incidence range of traumatic RRD has been consistently low (between 0.6–2 per 100,000 – see table 2.1).

2.3.2 Peripheral retinal degeneration and RRD

Areas of abnormal vitreo-retinal adhesion (oral bays, meridional complexes, retinal tufts) can predispose to retinal breaks during PVD, however efforts at characterising these lesions indicate they are uncommonly associated with RRD and carry a low risk of progression (<2%).(Ghazi & Green 2002) Similarly, most peripheral retinal degenerations are poorly characterised in epidemiology studies. The association with lattice degeneration is better known. Lattice degeneration is a vitreo-retinal degeneration of unknown pathogenesis. The prevalence increases with myopia from 4.3% in emmetropia to 14% in myopia of >-3 diopters (D).(Burton 1989) RRD patients with lattice are younger and more myopic than those without. Of Swedish patients with RRD and lattice (29.7%), one quarter had a refractive error of >-5D compared with 15.7% in eyes without lattice. (Tornquist *et al.* 1987a) A clinical report from India showed that 26.8% of eyes had lattice and a similar proportion were also myopic (31.3%).(Jalali *et al.* 2005) A higher proportion of lattice degeneration was found in Japanese studies (60-65%) with refractive error in these cases showing a myopic deviation away from the mean of >2D.(Sasaki *et al.* 1995)'(Shimizu H 1989) Depending on population characteristics, lattice degeneration appears to be an important factor associated with RRD in myopic eyes.

2.3.3 Macular status

Macular attachment at presentation is an important prognostic indicator of visual outcome. Western studies support a macula off detachment rate of 40-60%(Laatikainen & Tolppanen 1985a; Rowe *et al.* 1999), compared to a higher rate of 86.8% in developing countries.(Jalali *et al.* 2005) Schepens noted a macula off presentation in 65.1% of phakic RRDs in 1973, compared to a largely unchanged rate of 69% in the UK in 1997.(Ashrafzadeh *et al.* 1973)′(Sullivan PM *et al.* 1997)

2.3.4 Refraction and RRD

Myopia and especially high myopia are major risk factors for RRD.(C.L.Schepens 1961; Cambiaggi 1964; Druault-Toufesco S 1922) Independent of age, an eye with a spherical equivalent refractive error of -1 to -3D has a fourfold increased risk of RRD compared to an emmetropic eye, increasing to tenfold with a refractive error of >-3D. (Austin *et al.* 1990; The Eye Disease Case-Control Study Group 1993) Myopia predisposes to RRD through increased vitreous liquefaction, earlier PVD and higher incidence of vitreo-retinal degeneration.(Ghazi & Green 2002) Myopic patients are more likely to have RRD at a younger age and bilateral RRD.(Burton *et al.* 1983; The Eye Disease Case-Control Study Group 1993; Burton 1989; Li 2003; Yonemoto *et al.* 1994)

In Scandanavia, myopic detachments (>-2D) composed 40% and 50.3% of all phakic detachments in young age groups. This was 4-6 times higher than myopia prevalence in contemporary estimates. (Laatikainen *et al.* 1985)′(Tornquist *et al.* 1987a) In Croatia 46.9% of all phakic detachments were myopic (>-0.75D) compared with 22.8% of the general population. (Ivanisevic *et al.* 2000) A higher rate of low myopia (66-68%) was seen in Beijing and Shanghai.(Zou *et al.* 2002; Li 2003) In New Zealand, myopia of >-6D increased the risk of early RRD by 90%.(Polkinghorne & Craig 2004b)

The strong association between myopia and RRD in young age groups is evident, however ethnic differences are likely to underlie the reported variation, as myopia prevalence in Asian countries is known to be 1.5 to 2 fold higher than Caucasian populations of similar age. (Ling SK *et al.* 1987)'(Sperduto RD *et al.* 1983; Sasaki *et al.* 1995)

2.4 Variation, bias and the need for further research

The overall variation reported in these studies is large and likely to be due to differences in study design and population examined. However, this variation persists when examining non-traumatic phakic cases only and in reports from regions of comparable myopia prevalence and elderly population distribution, suggesting a potential (undiscovered) underlying influence. The methodology employed in 8 of 14 studies described were retrospective reviews, which may provide an accurate incidence estimate of the population studied, but are often limited by the precision of diagnostic and operational coding. While studies examined define RRD clinically, their inclusion with regard to re-operations, trauma, non-operated cases and out-patient cases differ. No studies have attempted to apply capture-recapture methodology to estimate under-ascertainment rates or have estimated the proportion of non-operated or out-patient cases. Similarly, few recent estimates of RRD incidence have used large samples sizes; most reporting < 500 cases. A detailed national or international register may allow for longitudinal studies and a more inclusive estimate of RRD incidence and its' variation over time. As out-patient procedures and day-case surgery become increasingly widespread, reliance on in-patient operative records to estimate RRD incidence are likely to become insufficient. A clear consensus on case definition would permit better comparison of data between studies.

2.5 Clinical characteristics of RRD

In this section I review the characteristics of RRD presented both in previously published epidemiology studies as well as in natural history studies.

2.5.1 Cataract extraction and pseudophakia

The risk of RRD after cataract extraction varies with the surgical method employed. This is due to a higher rate of PVD after cataract extraction and a lower hyaluronic acid concentration causing vitreous collapse.(Boberg-Ans *et al.* 2003; Ghazi & Green 2002) The cumulated six year risk of RRD after cataract surgery is increased by a factor of 6 to 8, increasing linearly for 20 years.(Javitt *et al.* 1994; Norregaard *et al.* 1996)'(Boberg-Ans *et al.* 2006)'(Erie *et al.* 2006) In the 1950s clinical series found a quarter of all RRDs were aphakic.(Ashrafzadeh *et al.* 1973) Over time the reported proportion of aphakia has declined, (42.7% in 1976; 18.6% in 1981; 4.7% in 1990; < 10% in 2000) (Haimann *et al.* 1982a; Tornquist *et al.* 1987a; Sasaki *et al.* 1995; Li 2003) reflecting cataract surgery trends.

Studies from Minnesota revealed 26.6% and 19% of RRD cases had previous cataract surgery.(Wilkes *et al.* 1982)'(Rowe *et al.* 1999) Between 1971-81, 18.6% of RRD cases had prior cataract extraction in Sweden. Twenty years later, the Swedish RRD register reported that 30.8% of over 1,000 detached eyes had prior cataract surgery.(Algvere *et al.* 1999; Tornquist *et al.* 1987a) By contrast, the combined proportion of aphakia and pseudophakia in China has been reported as only 10%,(Li 2003) and further UK and South American studies have supported this observation.(Mowatt *et al.* 2003; Limeira-Soares *et al.* 2007) More recently, a New Zealand study reported a

high rate (33%)of pseudophakia.(Polkinghorne & Craig 2004b) As a consequence of changing trends in cataract surgery and poorly reported rates, an accurate comparison of the incidence of pseudophakic RRD in previous studies is difficult. However, of the available data, reports in the last decade indicate a slightly higher incidence of pseudophakic RRD than the preceding decade. (see Table 2.1)

2.5.2 Increased rate of cataract surgery

Higher life expectancy and advances in cataract surgery has seen the proportion of pseudophakic individuals in developed countries rise. The Rochester Epidemiology Project reported a linear rise in cataract surgery rates between 1980 and 1994.(Baratz *et al.* 1997) The estimated prevalence of unilateral pseudophakia in North Carolina increased from 7.6% to 9.8% between 1998 and 2002, with the prevalence of bilateral pseudophakia more than doubling.(Williams A *et al.* 2006) Between 1986 and 1990 the number of cataract operations in Sweden rose from 227 to 328 per 100,000 of population and in the UK, the rate of cataract surgery increased 3.7-fold between 1989 and 2004.(Ninn-Pedersen *et al.* 1994; Keenan *et al.* 2007a)

The overall incidence of RRD has not shown this linear rise, however, the proportion of pseudophakia in RRD series appears to be increasing. A vitreo-retinal centre in London analysed cases of RRD in 1980 and again in 1999 finding that pseudophakia rose from 0.8% to 24%. Other UK reports support this large increase.(Minihan M. *et al.* 2001; Ah-Fat *et al.* 1999)In a series of 4,203 primary RRD cases in France the proportion of pseudophakia rose from 13% to 33% between 1989 and 2000.(Ducournau D.H. & Le Rouic JF. 2004) Strikingly, a comparison between the proportion of pseudophakic RRD in Minnesota between 1970 -78 and 1976-95 showed no significant difference, despite a four-fold rise in the rate of cataract extraction between

1980-94 in the same region.(Rowe *et al.* 1999; Wilkes *et al.* 1982; Baratz *et al.* 1997)

2.5.3 Type and frequency of retinal break

Tractional, PVD induced retinal tears or atrophic retinal holes can cause RRD.(Ashrafzadeh *et al.* 1973) Clinical studies observe a higher frequency of tractional tears in phakic RRD: India(186/395 vs 116/395)(Jalali *et al.* 2005), America (880/1,353 vs 578/1,353)(Ashrafzadeh *et al.* 1973), Helsinki (217/352 vs 61/352)(Laatikainen & Tolppanen 1985a) Round hole detachments occurred mainly in myopic eyes with lattice degeneration. Multiple breaks were more likely in young eyes with lattice degeneration. (Laatikainen & Tolppanen 1985b)Tears were more frequent in non-myopic or moderately myopic phakic patients over forty.(Tornquist *et al.* 1987b) In two US studies, atrophic holes accounted for 3% and 14% of detachments.(Morse & Scheie 1974; Tillery WV & Lucier AC 1976) In Japan, of patients with lattice degeneration, 40% of RRDs were caused by tractional tears and 20% by atrophic holes. A Finnish study stratified breaks by age of onset noting that in the under 20 year olds, dialysis was the most common, between 20 and 40 years horse-shoe tear and round holes were equally common and over the age of 40, the most common causative break was a PVD associated horse-shoe shaped tear. (Sasaki *et al.* 1995; Laatikainen & Tolppanen 1985b) Giant retinal tears and retinal dialysis are uncommon, accounting for 0.6%-7.7% of phakic RRD.(Ashrafzadeh *et al.* 1973; Laatikainen & Tolppanen 1985b; Jalali *et al.* 2005; Sasaki *et al.* 1995) Macular hole RRD is uncommon in European(Laatikainen & Tolppanen 1985a) and American(Margherio RR & Schepens CL 1972) series (0.5% and 1.7%) but accounts for 8.9% and 21.1% of RRDs in more myopic series.(Sasaki *et al.* 1995)'(Zhang & Hu 1982)

2.5.4 Area of detachment and location of retinal breaks

Clinical series have found that the most frequent site of peripheral retinal tears are in the supero-temporal quadrant, followed by the infero-temporal quadrant. Taking these into account, between 61.6-96% of horse-shoe shaped retinal tears are found in the temporal retina. Round holes are more commonly associated with young age, myopia and lattice degeneration and are commonly in the infero-temporal region. One large series noted that HST in high myopia is often found in the supero-nasal quadrant. (Laatikainen & Tolppanen 1985b; Tornquist *et al.* 1987b)Retinal dialysis are often associated with trauma (>77%) and the majority are located in the infero-temporal quadrant.(Laatikainen & Tolppanen 1985b)A higher incidence of nasal retinal break have been found in clinical series examining aphakic retinal detachments.(Ashrafzadeh *et al.* 1973) Further clinical studies indicate that in between 52- 66% of cases, there are less than 2 quadrants (or six clock hours) of retina detached. Total RRD was noted to be present in 17.2%. Myopic RRD was often localised with a higher majority (75%) limited to <2 clock hours of detachment.(Laatikainen & Tolppanen 1985b; Tornquist *et al.* 1987b)

2.6 Bilateral RRD

Bilateral RRD affects between 3–33% depending on case inclusion and study duration.(Gupta & Benson 2005) Earlier large clinical studies noted a high rate (473/2,016 and 1,642/5,270) of bilateral RRD including re-detachments over ten years.(C.L.Schepens 1961; Ashrafzadeh *et al.* 1973) Over one year,

rates of bilateral RRD vary between 3.5 and 5.82%, (Sasaki *et al.* 1995; Li 2003; Polkinghorne & Craig 2004b; Haimann *et al.* 1982a) increasing to 9-10% over a four year period.(Laatikainen *et al.* 1985; Laatikainen & Tolppanen 1985a; Zou *et al.* 2002) In Sweden over ten years and Minnesota over 20 years, 11.2%(34/538) and 6%(18/293) of subjects had bilateral RRD.(Tornquist *et al.* 1987a; Rowe *et al.* 1999) In all series, RRD has affected a proportion of both eyes indicating over time fellow eye involvement is an important consideration.

Study Population and Publication Year	Region and Study Design	Study Type (Methodology Score)	Study Duration (years)	Total study population	No. of RRD cases	Total Incidence per 100,000 of population (95%CI)	Incidence per 100,000 of phakic non-traumatic RRD(95%CI)	Incidence per 100,000 of pseudophakic RRD (95%CI)*	Incidence per 100,000 of traumatic RRD (95%CI)	% Macula detached at presentation	% Myopic RRD cases	% Bilateral	Trauma included	Re-operations included
Singapore(Wong *et al.* 1999) 1999	Singapore	ICD-9 (15)	4	2,705,115	1126	10.5 (10.2-10.9)	N/S	N/S	N/S	N/S	N/S	N/S	N/S	No
Stockholm and surrounding counties(Algvere *et al.* 1999) 1999	Sweden	Prospective; diagnosis by clinician (12)	1	1,744,330	1116	14	N/S	N/S	N/S	52.3%	-4.16 (-5 to -3.3D)^	N/S	N/S	No
Orebro and Varmland(Tornquist *et al.* 1987a) 1987	Sweden	Prospective; diagnosis by clinician (16)	10	N/S	590	10.6(9.8-11.4)	7.97	1.97*	0.66	N/S	25% (>-2D)	11.2%	Yes	Yes
Beijing(Li 2003) 2003	China	Prospective; diagnosis by clinician (20)	1	6,589,000	526	7.98(7.3-8.67)	6.25(5.65-6.86)	0.8*(0.59-1.02)	0.93 (0.69-1.16)	N/S	66.5% (>-1D)	5.8%	Yes	No
Iowa County(Haimann *et al.* 1982a) 1982	U.S.A	Questionnaire survey (13)	1	2,824,500	361	12.4(11.5-14.2)	6.1(5.4-7.3)	N/S	1.4(1-2)	N/S	N/S	3.4%	Yes	N/S
Sao Paolo(Limeira-Soares *et al.* 2007) 2007	Brazil	ICD-10 (16)	1	3,389,294	313	9.2(8.2-10.3)	7.1(6.3-8.1)	1(0.7-1.4)	0.9(0.6-1.3)	N/S	N/S	N/S	Yes	No
Olmstead County, Minnesota(Rowe *et al.* 1999) 1999	U.S.A	ICD-9 (15)	20	106,470	311	17.9(15.9-19.9)	12.6(10.9-14.3)	3.4(2.46-4.16)*	1.3(0.8-1.7)	41%	N/S	6%	Yes	N/S
Helsinki(Laatikainen *et al.* 1985) 1985	Finland	Retrospective assessment (16)	4	1,121,955	310	6.9(5.5-8.7)	5.4(4.3-7)	N/S	0.8(0.4-1.6)	56.5%	40% (>-1D)	9.9%	Yes	N/S
Split-Dalmatia County(Ivanisevic *et al.* 2000) 1999	Croatia	Retrospective assessment (7)	11	465,947	272	N/S	5.3(4.1-6.2)	N/S	N/S	N/S	46.9% (>-0.75D)	2.2%	No	N/S
Kumamoto(Sasaki *et al.* 1995) 1995	Japan	Retrospective assessment (17)	1	1,840,300	192	10.4(9-12)	9.8(8.5-11.4)	N/S	0.2(0-0.5)	N/S	-3.7 +/- 5D^^	4.2%	Yes	N/S
Auckland(Polkinghorne & Craig 2004b) 2004	New Zealand	Prospective; diagnosis by clinician (20)	1	1,205,694	146	11.8(9.8-13.7)	6.1(4.7-7.6)	4.1(3-5.4)	2(1.3-3)	N/S	23% (>-6D)	3.5%	Yes	No
Wolverhampton(Mowatt *et al.* 2003) 2003	U.K.	Retrospective assessment (15)	5	248,600	140	11.3(9.48-13.3)	9.7(8-11.4)	1.2(0.68-2)	0.7(0.34-1.38)	N/S	N/S	N/S	Yes	N/S
Walsall(Mowatt *et al.* 2003) 2003	U.K.	Retrospective assessment (15)	5	259,517	83	6.3(5.1- 7.92)	5.4(4.28-6.9)	0.9(0.48- 1.62)	0.5(0.22-1.12)	N/S	N/S	N/S	Yes	N/S
Shanghai(Zou *et al.* 2002) 2002	China	Prospective; population survey (13)	4	108,132	61	14.4(10-18.6)	N/S	N/S	6.5(2.6-13.3)	N/S	68.8% (>-3D)	9.8%	Yes	No
Rochester, Minnesota(Wilkes *et al.* 1982) 1982	U.S.A	Retrospective assessment (14)	8	494,505	45	9.1(6.6-12.2)	N/S	N/S	N/S	N/S	35.5% (>-1D)	6.7%	No	No

* Includes aphakic cases
N/S – Not specified
^Mean refraction of all cases under 50 years (95% CI)
^^Mean refraction and SD of all cases

Table 2.1 –Reported incidence of RRD (per 100,000 of population), clinical characteristics and inclusion criteria as reported in previous population based epidemiology studies ordered by sample size.

Study Location	**Incidence of RRD per 100,000 of population**		**Percentage of total population over 60 years**
	Age group: 60-69 years (95% CI)	**Age group: 70-79 years (95% CI)**	
Singapore(Wong *et al.* 1999)	21.9 (19-24.8)		9.1%
Orebro, Sweden(Tornquist *et al.* 1987a)	28		23%
Beijing, China(Li 2003)	22.15 (18.41-25.89)	15.21 (10.82-19.61)	14.9%
Iowa, U.S.A(Haimann *et al.* 1982a)	19.2	24.2	16.9%
Brazil(Limeira-Soares *et al.* 2007)	49.9	38.7	8.9%
Olmsted County, Minnesota, U.S.A(Rowe *et al.* 1999)	70	50	N/S
Finland(Laatikainen *et al.* 1985)	21.8		15.2%
Croatia(Ivanisevic *et al.* 2000)†	22.1	15.8	15.8%
Japan(Sasaki *et al.* 1995)	22.7	18.9	22%
New Zealand(Polkinghorne & Craig 2004b)	49.7	32.6	13.8%
West Midlands, U.K.(Mowatt *et al.* 2003)	Wol 27.4 Wal **42.4	29.1 ***69.5	39.9%Δ
Shanghai, China(Zou *et al.* 2002)	17.5 (12.9-22)		18.9%
Rochester, Minnesota, U.S.A(Wilkes *et al.* 1982)	*31.3		N/S

Wol – Wolverhampton
Wal - Walsall
*65+ yrs
** 65-74 yrs

***75-84 yrs
Δ 40-79 yrs
†Non-traumatic phakic only
N/S – Not specified

Table 2.2 –Age specific incidence of RRD in over 60 year olds as reported in previous epidemiology studies (ordered by sample size) and the proportion of study population over 60 years.

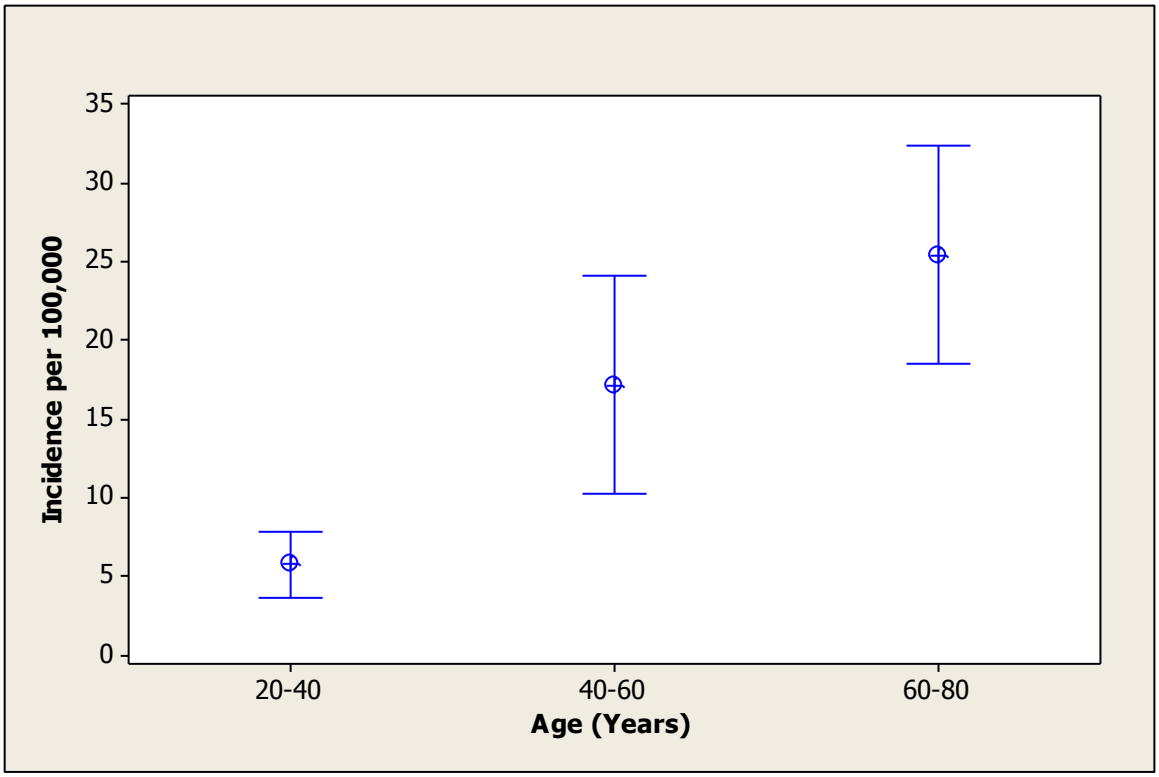


Figure 2.4 – Mean and 95% confidence intervals of RRD incidence in previous population based estimates by age group.

CHAPTER 3 Temporal trends in the rate of RRD and cataract surgery in Scotland

3.1 Introduction

An accurate epidemiology study requires high case ascertainment from a stable, well defined population that actively seeks medical care. Because of this, few studies have systematically examined the changes and influences on the incidence rate of RRD across different time periods in a well defined geographic area. There are six vitreo-retinal surgical sites in Scotland which are responsible for diagnosing and managing all RRD cases (Ayr, Glasgow, Edinburgh, Dundee, Aberdeen and Inverness). The stable population structure and well defined referral and treatment patterns make Scotland an ideal region to conduct an epidemiology study.

This chapter examines the trends in RRD incidence in Scotland between 1986 and 2007, taking into account changes in the size and composition of the population and examines the changes in cataract surgery rates over this period.

3.2 Methodology

3.2.1 Data Source-Information Services Division Scotland

The Information and Statistics Division of the Scottish Executive (ISD) is Scotland's national organisation for health information and statistics. They are responsible for National Health Service (NHS) hospital episode statistics data which comprise administrative, demographic and medical information on all inpatient and day-case procedure hospital episodes in all general and acute wards in Scotland. Private patients are not included in this database.

These data are collected in hospital by trained clerical staff who assign diagnostic codes for each admission at the time of patient discharge. The information is transferred to the Information and Statistics Division of the Scottish Executive who collate and analyse the data.

Since 1987 these data have been linked to successive episodes of care for each person, so that individuals can be traced through multiple episodes of care. This record linkage is done through a probability matching algorithm. Firstly, to determine which record pairs belong to the same person, the records are matched by a Soundex/NYSIIS code (a name compression algorithm), first initial, sex, and date of birth. If there is a discrepancy in any one of these, the records will not be matched. Secondly, probability weights are calculated and applied to determine the likelihood that the records are from the same individual. This logarithmic weighting is based upon demographic criteria including surname, maiden name, sex, date of birth, residential postcode and other correspondence criteria such as date of admission and date of discharge. Once the probability weights are ordered, the threshold (defining linkage) is set, usually at the 50/50 point. This is an automated process, with larger groups of records contributing to a higher false positive rate. Clerical monitoring of record pair batches estimate both the false positive and false negative rates from this process to be approximately 3%.(2008)

We obtained all first diagnosis of RRD and first operation for cataract for patients in Scotland between 1987 and 2006 based on diagnostic and procedural coded data derived from the ISD. The diagnostic codes used to identify patients with RRD were 361.0 – 361.9 and 362.4 in the International Classification of Disease 9th edition (ICD-9) and H33.0 -33.5 in ICD-10. The operative codes used to identify patients undergoing cataract surgery from

were 173 to 178 in the Office of Population, Censuses and Surveys: Classification of Interventions and Procedures, 3rd edition (OPCS-3) and C71-C75 in OPCS-4. Over the analysis period, coding of RRD changed between ICD-9 and ICD-10, however no corresponding change in hospital admission rates was noted during this changeover period (between 1994 and 1995). Similarly, the OPCS procedural coding system has undergone several revisions, but no corresponding significant or stepwise change in CSR was noted. (Table 3.2a+b)

3.2.2 Statistical Methods

I first used a log-linear regression model to examine the trends in RRD incidence and the cataract surgery rate over time, and calculated an average annual percent change (AAPC) in incidence rate over the study period. I added a quadratic trend term ($year^2$) to examine for possible non-linear trends in RRD incidence.

I then used age-period-cohort modeling to explore the effects of chronological age, time period, and birth cohort on RRD incidence trends. Individual data was grouped into eighteen 5-year age groups from 0-4 years through to 85+ years, four 5-year calendar periods from 1987-1991 through to 2002-2006 and twenty derived birth cohorts. Assuming a Poisson distribution of cases of RRD, a log-linear regression model was used to estimate the changes in RRD incidence by age, period and birth cohort. (R v2.10.1) The Poisson model used took the form of:

$$-\log(\text{rate}) = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}$$

Where α_i is the age effect, β_j is the period effect and γ_k is the cohort effect. The term ε_{ijk} is the random error term. The parameter estimates were

calculated as maximum likelihood estimates. The first period (1987-1991) and last age group (85+) were assigned as reference groups. Based on this general form, I examined 5 models in sequence: a one-factor age model, a two-factor age-drift model, age-cohort model, age-period model, and an age-period-cohort model. The drift term in the age-drift model represents a temporal change in incidence rate not identifiable as a period or cohort effect. I calculated goodness of fit (R-squared) statistics based on model differences in deviance and degrees of freedom, to determine the model accounting for the most variability. The full age-period-cohort model had the lowest residual deviance and an R^2 of 0.96 and was used in the analysis.(Table 3.1)

Age and sex-specific incidence rates of patients admitted with a first diagnosis of RRD and those undergoing cataract surgery in Scotland were calculated annually. Annual incidence rates were calculated on the basis that the first admission only counted toward the episode rate, so that recurrent individual admissions with the same diagnostic code were not counted after 12 months from the first admission. This method aims to eliminate re-operations on RRD cases; however, consecutive bilateral RRD cases will only have been counted once. Age and sex standardised rates were calculated by a direct method using the European Standard Population as a reference. Annual population data was obtained from the General Register Office for Scotland from the Scottish population censuses since 1987. Using the 1987 age specific RRD incidence rate and the 2006 population census, I calculated the expected standardised incidence rate for each age group in 2006 and compared this to the observed incidence rates in 2006.

3.2.3 Data Quality

Retrospective hospital episode statistics data which I have used to estimate disease incidence have some limitations,(2007a; Milburn *et al.* 2007) including insufficient clinical details, incorrect diagnostic coding, duplicate entries and incomplete population coverage. Thus the use of HES to estimate disease incidence is by necessity an approximation and often limits the ability of the investigator to examine other influencing aspects of disease, such as in this case, the prevalence of myopia or ocular trauma. However HES remain a useful indicator of changing incidence trends, particularly where the study population is a stable one actively seeking medical care and the disease under investigation requires hospital admission for treatment.

The ISD Data used in this study is derived from hospital episode statistics, the accuracy of which is dependent on appropriate and exact coding. A recent quality control audit of surgical specialties (excluding general surgery) indicates coding for main condition is accurate in 88.5% (95%CI:86.3-90.7) and for main operation in 93% (95%CI: 91.2-94.8).(2007a) Inappropriate record linkage of hospital episode statistics can be up to 3%.(2008) The proportion of RRD cases treated in private sector is unknown, however I expect this number to be very low, as elective eye operations in England and Wales in 1998 (excluding cataract extraction) accounted for only 1.8% of all eye operations performed in NHS hospitals.(Williams *et al.* 2000)This relevant proportion is likely to be lower in Scotland as RRDs diagnosed and followed-up privately will be operated on in NHS hospitals.

3.3 Results

3.3.1 Temporal trends in cataract surgery in Scotland

The annual and age standardised cataract surgery rate (CSR) in Scotland between 1987 and 2006 are shown in figure 3.1 and 3.2. The CSR in Scotland rose from 145 per 100,000 in 1987 to 575 per 100,000 in 2006, representing an overall increase of 296%. There was no significant gender difference in the increase in CSR over the calendar years. Figure 3.3a and 3.3b demonstrate a significant and dramatic rise in CSR in males and females aged over 60 years across 4 time periods in Scotland. From the age of sixty years and onwards, there was a steady rise in the CSR, with a peak for both genders in the 80-89 year age group. Women had a higher overall CSR than men across 4 time periods. Using a linear regression model, the average annual percent change in each health board region in Scotland was calculated. (Table 3.3) Across all health board regions combined, there was an average annual increase in CSR of 6.6% (95%CI 6.08-7.14), with virtually all regions demonstrating a significant annual increase of between 5 to 11% between 1987 and 2006.

3.3.2 Temporal trends in RRD incidence in Scotland

The age specific and age standardised annual RRD incidence rate between 1987 and 2006 is shown in table 3.4. (Table 3.5a(males); Table 3.5b(females)) The crude incidence rate of RRD for all ages and both genders rose steadily from 10.06 per 100,000 (95%CI: 9.19-10.93 per 100,000) in 1987 to 15.28 per 100,000 (95%CI: 14.21-16.35 per 100,000)

in 2006. The incidence of RRD was higher in males of all age groups, and the temporal rise in incidence was more marked in males. The age standardised male:female incidence ratio rose from 1.40 in 1987 to 1.76 in 2006.($p<0.001$) Figure 3.4 demonstrates the age standardised incidence and associated 95% confidence intervals in men and women. Figure 3.5(a+b) and figure 3.6 (a+b) demonstrate the age and sex specific RRD rates for men and women between 1987 and 2006. Both genders showed a significant rising trend in the highest incidence age groups (60-79 years). In men, the strongest rising trend in incidence was found in younger age groups between 40 to 59 years, a pattern which was absent in females.

The average annual percent change (AAPC) in RRD incidence increased annually by 1.9% overall in the 20 year period. Significant increases were noted in nearly all age groups with the exception of those under 20 years and in the 30-39 year age group. (Table 3.6) The second order quadratic trend term did not demonstrate a significant change in incidence, with the exception of the over 80 year age group.

I calculated the expected age specific incidence rate in 2006 using the 1987 age specific rate standardised to the 2006 Scottish population and compared this to the observed age specific rate in 2006. (Table 3.7) The observed rate in 2006 was significantly higher than the expected rate in all age groups over 40 years.

Figure 3.7 demonstrates the combined age-period-cohort effects on RRD incidence. Chronological age demonstrates a bimodal distribution in incidence with a peak in the sixth decade, as well as a secondary smaller peak in the 3rd decade. No significant period effects were found. No significant birth cohort effects were found in cohorts since 1940. Prior to this there was a reduction in risk ratio (0.25-0.86) as fewer parameters were available for analysis.

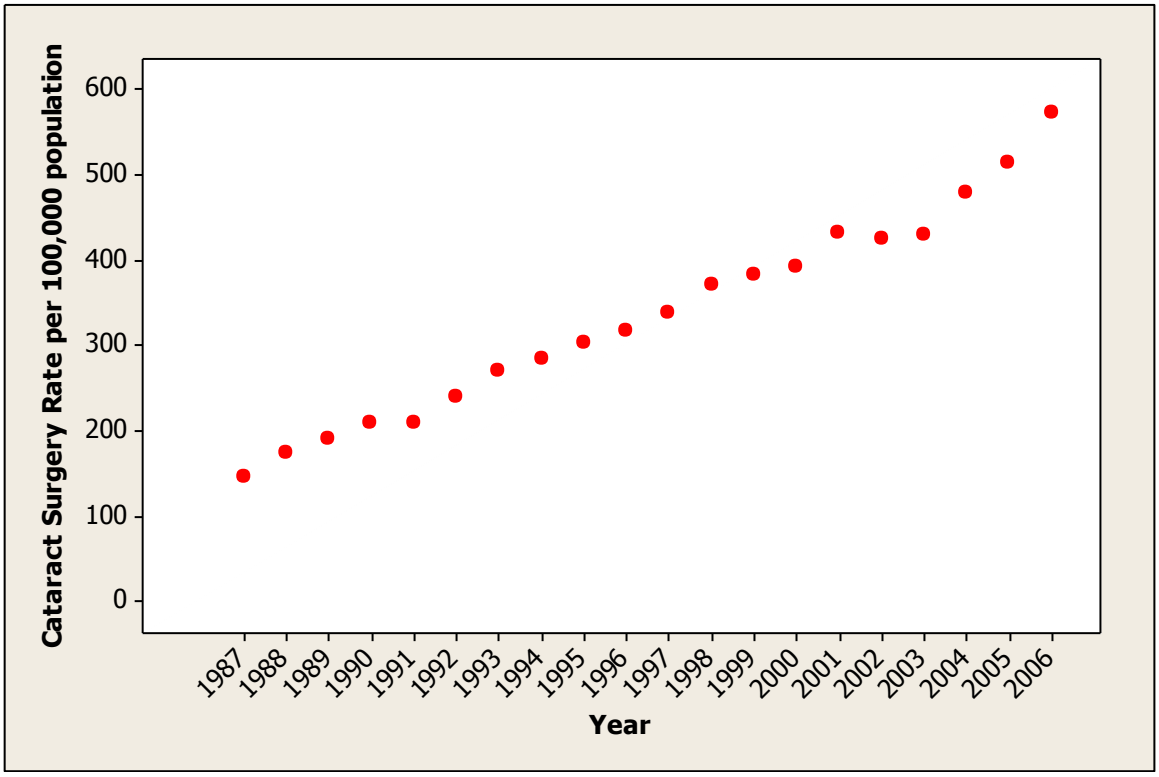


Figure 3.1 – Annual cataract surgery rates (CSR) in Scotland between 1987 and 2006 per 100,000 of population

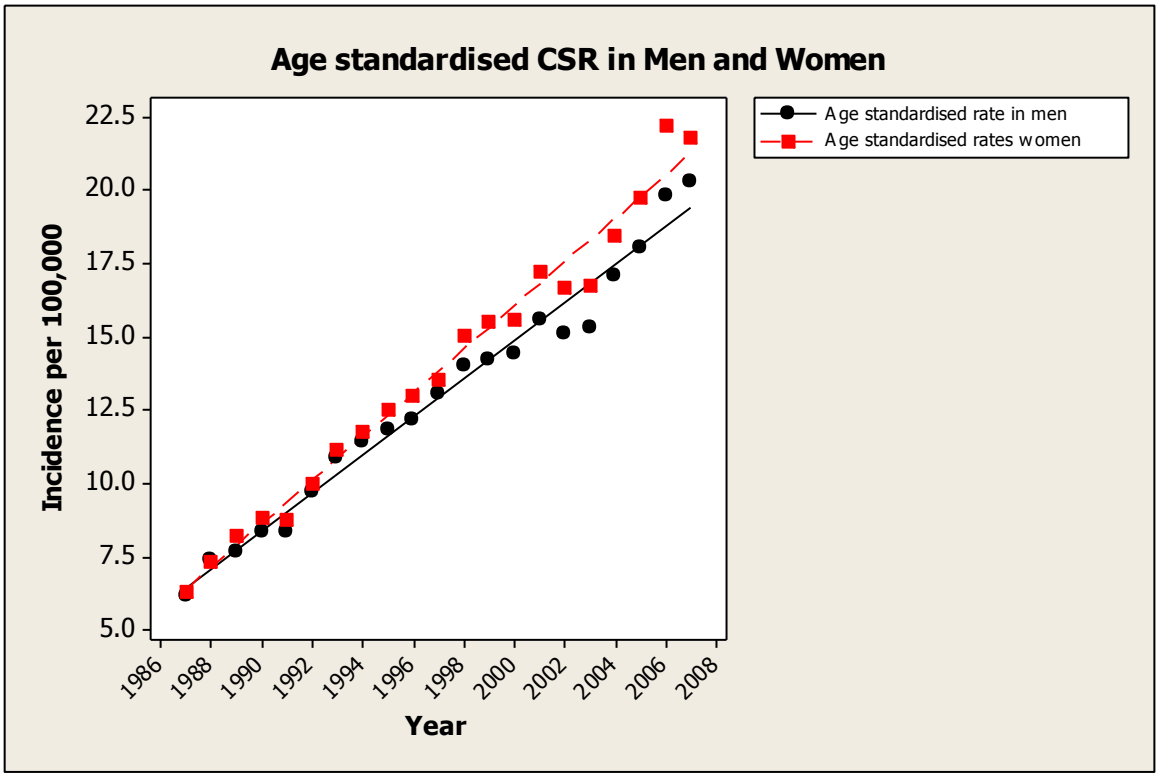


Figure 3.2 - Age standardised (direct method) CSR in men and women between 1987 and 2006 demonstrating a linear increase in CSR. No significant difference was found in the rate of rise between the sexes. (p=0.5)

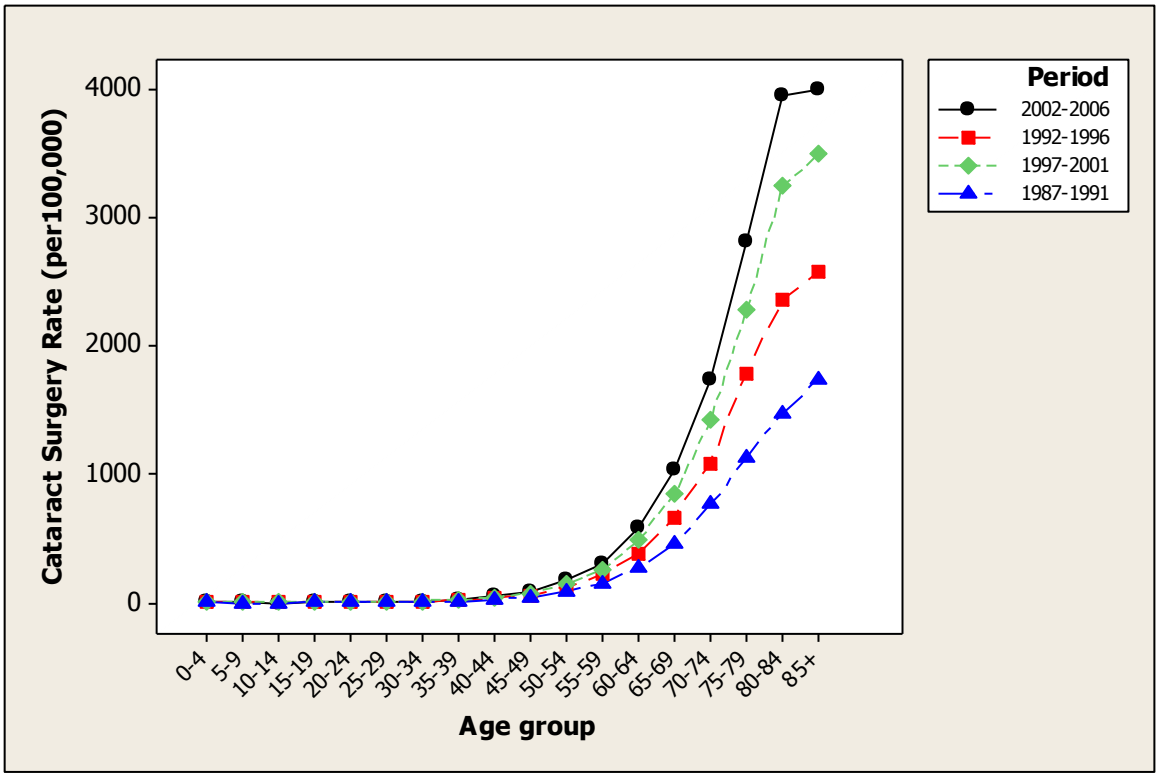


Figure 3.3a – Age specific mean cataract surgery rate in men over 4 time periods.

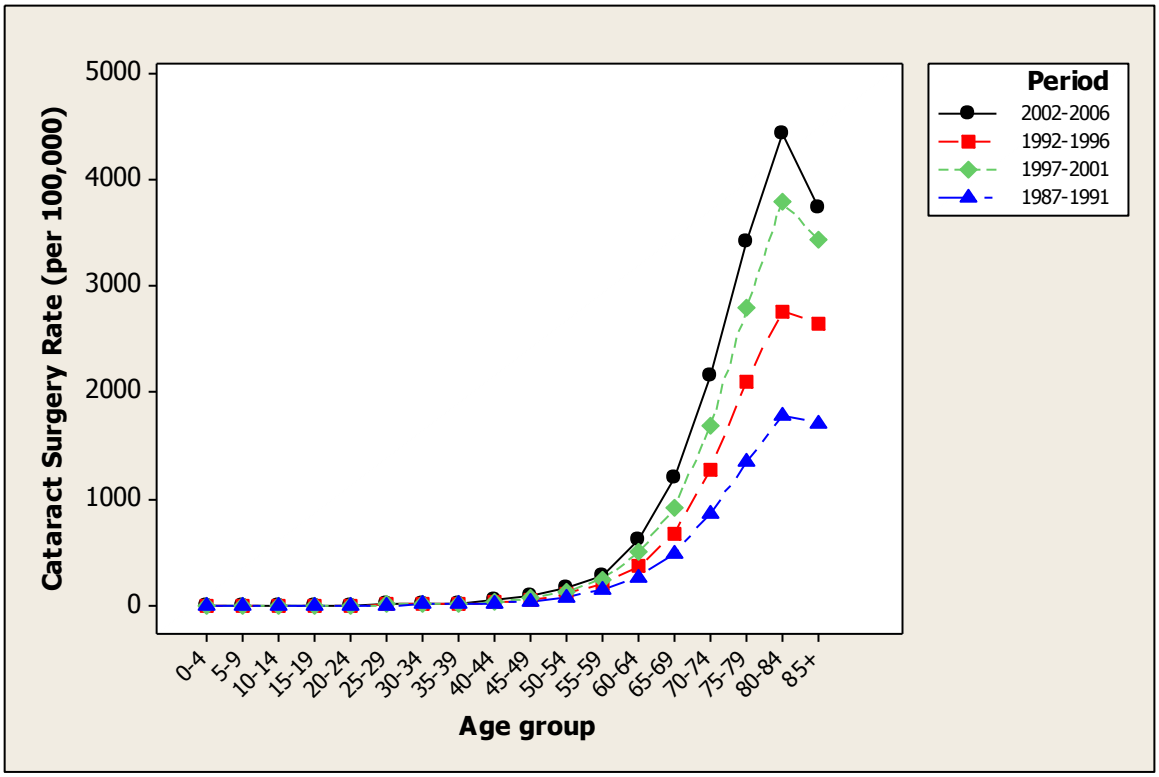


Figure 3.3b – Age specific cataract surgery rate in women over 4 time periods.

	Residual Deviance(Df)	Deviance (Df)	P- value	Adjusted R-squared
Age	1291.37(126)			
Age-drift	1216.9(125)	74.47(1)	< 0.001	
Age-Cohort	1039.82(106)	177.08(19)	<0.001	0.87
Age-Period	1214.19(123)	2.71(2)	0.25	0.98
Age-Period-Cohort	1034.1(104)	5.72(2)	0.044	0.96

Table 3.1 – Summary statistics comparing the goodness of fit for different age-period-cohort models used to examine changes in RRD incidence.

OPCS 4	OPCS 3
C71.1 Extracapsular extraction of lens simple linear extraction of lens	173 Extra-capsular Extraction of Cataract (no further subdivisions)
C71.2 Extracapsular extraction of lens phakoemulsification of lens	173 (no further subdivisions)
C72.1 Intracapsular extraction of lens forceps extraction of lens	1741 Intra-capsular forceps extraction of cataract
C72.2 Intracapsular extraction of lens suction extraction of lens	1744 Intra-capsular suction/ventouse/erisiphake extraction of cataract
C72.3 Intracapsular extraction of lens cryoextraction of lens	1745 Intra-capsular cryo-extraction of cataract
C72.8 Intracapsular extraction of lens other specified	1742, 1743 Intra-capsular linear (1742) and chymotrypsin (1743) extraction of cataract
C72.9 Intracapsular extraction of lens unspecified	174 Intra-capsular extraction of cataract
C74.1 Other extraction of lens curettage of lens	176 Other extraction of lens, not elsewhere classified (no further subdivisions)
C74.2 Other extraction of lens discission of cataract	176 (no further subdivisions)
C74.3 Other extraction of lens mechanical lensectomy	176 (no further subdivisions)
C74.8 Other extraction of lens other specified	176 (no further subdivisions)
C74.9 Other extraction of lens unspecified	176 (no further subdivisions)
C75.1 Prosthesis of lens insertion of prosthetic replacement for lens	1781 Insertion of intra-ocular aphakia implant
C75.2 Prosthesis of lens revision of prosthetic replacement for lens	no equivalent code
C75.3 Prosthesis of lens removal of prosthetic replacement for lens	1782 Removal of Intra-ocular aphakia implant
C75.8 Prosthesis of lens other specified	178 Intra-ocular aphakia implant
C75.9 Prosthesis of lens unspecified	178 Intra-ocular aphakia implant

Table 3.2a – Selected codes for cataract surgery in the Office of Population, Censuses and Surveys: Classification of Interventions and Procedures (v3 and v4)

ICD - 10	ICD-9
H33.0 - Retinal Detachment with retinal break	361.0 - Retinal detachment with retinal defect
H33.1 - Retinoschisis and retinal cysts	361.1 - Retinoschisis and retinal cysts
H33.3 - Retinal breaks without detachment	361.3 - Retinal defects without detachment
H33.5 - Other retinal detachments	361.8 - Other forms of retinal detachment
Also consider:	361.9 - Unspecified
H35.7 - Separation of retinal layers	362.4 - Separation of the retinal layers

Table 3.2b – Selected codes for rhegmatogenous retinal detachment based on International Classification of Disease (ICD-9 and ICD-10).

Region	AAPC (%)	LOWER 95% CI	UPPER 95% CI	P-value
All Scotland	6.61	6.08	7.14	<0.001
Ayrshire	9.31	8.44	10.29	<0.001
Borders	8	5.02	10.51	<0.001
Dumfries and Galloway	11.07	9.97	12.12	<0.001
Fife	5.23	4.29	6.18	<0.001
Forth Valley	8.33	7.25	9.53	<0.001
Grampian	-2.18	-5.16	0.9	0.144
Glasgow and Clyde	6.61	6.18	7.14	<0.001
Highland	9.64	8.98	10.4	<0.001
Lanarkshire	7.68	6.6	8.65	<0.001
Lothian	5.97	4.5	7.46	<0.001
Orkney	7.36	5.34	9.41	<0.001
Shetland	5.87	4.19	7.47	<0.001
Tayside	5.55	4.92	6.18	<0.001
Western Isles	9.09	7.578	10.52	<0.001

Table 3.3 – Analysis of average annual percent change (AAPC) in cataract surgery rates by health board region in Scotland between 1987 to 2006.

(A)		Linear model		Quadratic trend term	
	Age	**AAPC (%)**	**p-value**	**Sign of 2nd order term**	**p-value**
	0-9	+1.1	0.588	+	0.066
	10-19	-1.2	0.166	+	0.194
	20-29	+1.1	0.051	-	0.176
	30-39	+0.2	0.619	+	0.782
	40-49	+1.2	0.04	-	0.047
	50-59	+2.6	<0.001	-	0.915
	60-69	+1.9	<0.001	-	0.474
	70-79	+1.6	0.001	-	0.951
	80+	+1.9	0.019	-	0.001
	All ages	+1.9	<0.001	-	0.334

Table 3.6 – Age specific average annual percent change (AAPC) of RRD incidence in Scotland between 1987-2006.

(B)		Incidence per 100,000 population (95% Confidence Interval)	
	Age	**Expected 2006 Incidence**	**Observed 2006 Incidence**
	0-9	0.91(0.3-2.1)	1.64(0.8-3.1)
	10-19	4.45(2.9-6.4)	3.14(1.9-4.8)
	20-29	6.92(5.1-9.3)	6.77(4.9-9.1)
	30-39	7.98(6-10.4)	9.12(7-11.6)
	40-49	7.40(5.6-9.6)	13.28(10.9-16.1)
	50-59	12.49(10-15.5)	27.64(23.8-31.9)
	60-69	22.94(19-27.4)	34.42(29.6-39.8)
	70-79	30.72(25.4-36.8)	35.18(29.5-41.7)
	80+	13.56(9.1-19.5)	18.24(13-24.9)
	All ages	10.61(9.7-11.5)	15.28(14.2-16.4)

Table 3.7 – The age specific expected and observed incidence of RRD in 2006 calculated using the 1987 age specific incidence standardised with the 2006 Scottish population estimate.

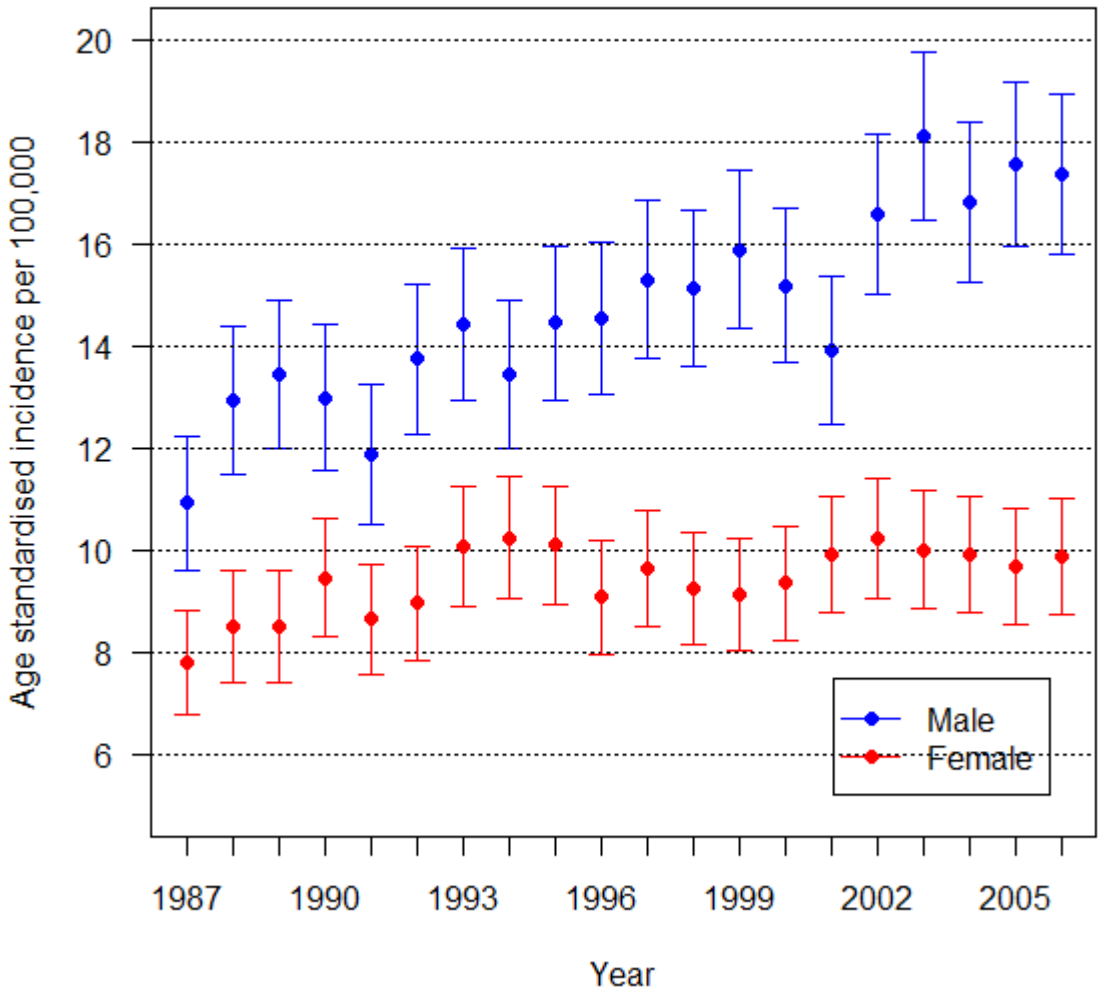


Figure 3.4– Age standardised incidence of RRD in men and women. A rising trend was noted for both genders, but was much stronger in men.

	Incidence per 100,000 population Age Group										Age standardised Incidence(100,000)		
Year of Diagnosis	**0-9**	**10-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**80+**	**All Ages (95%CI)**	**Men (95%CI)**	**Women (95%CI)**	**M:F Ratio**
1987	0.94	4.36	6.92	7.92	7.36	12.50	23.02	30.65	13.38	10.06 (9.19-10.93)	10.92 (9.61-12.23)	7.80 (6.77-8.82)	1.40
1988	1.09	5.84	5.28	8.24	10.42	19.33	25.24	23.08	16.68	11.01 (10.10-11.92)	12.93 (11.48-14.38)	8.49 (7.39-9.59)	1.52
1989	1.08	3.67	7.00	8.45	11.48	18.44	28.09	26.81	13.16	11.52 (10.59-12.45)	13.44 (11.99-14.90)	8.50 (7.41-9.59)	1.58
1990	0.93	2.70	7.03	8.87	11.15	19.93	28.12	28.91	17.09	11.85 (10.90-12.79)	12.98 (11.55-14.41)	9.45 (8.30-10.61)	1.37
1991	0.47	3.69	5.49	8.43	10.20	16.47	28.95	25.39	17.86	11.00 (10.09-11.91)	11.87 (10.51-13.23)	8.64 (7.55-9.73)	1.37
1992	0.62	3.60	7.17	9.21	12.96	17.21	28.78	27.11	17.52	11.94 (10.99-12.89)	13.73 (12.27-15.19)	8.96 (7.84-10.08)	1.53
1993	1.08	3.33	6.91	9.68	10.89	21.36	34.51	28.53	23.34	13.06 (12.07-14.05)	14.42 (12.92-15.92)	10.08 (8.90-11.26)	1.43
1994	0.62	3.50	7.58	8.19	10.92	20.43	30.92	35.81	14.27	12.64 (11.67-13.62)	13.43 (11.99-14.87)	10.24 (9.05-11.43)	1.31
1995	0.16	3.18	7.41	8.68	13.98	21.04	30.40	32.59	22.07	13.11 (12.11-14.10)	14.44 (12.95-15.94)	10.09 (8.92-11.26)	1.43
1996	0.79	4.13	6.15	10.27	11.94	21.24	26.51	28.07	22.57	12.49 (11.52-13.56)	14.53 (13.04-16.03)	9.07 (7.95-10.19)	1.60
1997	0.96	3.62	8.99	7.43	13.09	21.14	28.72	35.40	22.20	13.36 (12.35-14.36)	15.30 (13.76-16.83)	9.63 (8.49-10.78)	1.59
1998	0.65	3.43	7.43	8.16	11.90	20.11	35.58	27.55	24.65	13.20 (12.20-14.20)	15.13 (13.61-16.65)	9.24 (8.13-10.35)	1.64
1999	1.48	3.11	9.18	8.67	12.51	19.72	29.54	32.99	26.04	13.53	15.88	9.13	1.74

										(12.51-14.54)	(14.32-17.44)	(8.02-10.24)	
2000	0.67	2.34	6.72	8.23	12.49	23.22	30.49	30.58	24.99	13.33 (12.33-14.34)	15.17 (13.66-16.69)	9.35 (8.22-10.47)	1.62
2001	0.52	2.34	8.09	6.37	9.62	20.75	35.53	34.07	22.06	13.21 (12.21-14.21)	13.91 (12.46-15.36)	9.92 (8.77-11.06)	1.40
2002	0.88	4.99	7.27	9.58	12.86	25.00	34.31	31.86	24.02	14.86 (13.79-15.92)	16.56 (14.99-18.13)	10.22 (9.05-11.38)	1.62
2003	1.26	3.57	7.33	9.63	13.55	26.89	38.25	32.54	19.30	15.42 (14.34-16.51)	18.10 (16.45-19.74)	10.00 (8.84-11.16)	1.81
2004	0.54	3.71	9.24	8.23	10.51	23.54	35.92	39.33	22.35	14.97 (13.90-16.03)	16.81 (15.24-18.38)	9.91 (8.77-11.04)	1.70
2005	1.09	3.27	6.98	9.72	11.61	27.55	36.27	34.06	16.75	15.07 (14.01-16.14)	17.57 (15.96-19.17)	9.67 (8.73-11)	1.82
2006	1.64	3.14	6.78	9.12	13.28	27.64	34.42	35.18	18.25	15.28 (14.21-16.35)	17.35 (15.77-18.93)	9.87 (8.83-11.11)	1.76

Table 3.4 – The annual incidence of RRD by age group and the age standardised incidence in men and women between 1987-2006. This table highlights the increasing incidence of RRD and demonstrates the much higher age standardised incidence in men.

MALE	Incidence per 100,000 Age Group									
	0-9	**10-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**80+**	**All Ages (95% CI)**
Year										
1987	0.91	5.89	8.97	8.94	9.60	17.49	21.26	35.93	9.33	11.12 (9.80-12.44)
1988	1.51	8.95	5.95	9.29	12.96	22.34	29.02	30.93	22.46	12.85 (11.43-14.27)
1989	1.81	5.19	9.59	12.69	14.61	18.60	34.76	30.03	12.94	13.63 (12.17-15.09)
1990	1.21	4.13	10.64	10.79	14.38	20.85	32.20	25.66	23.16	13.26 (11.81-14.70)
1991	0.91	6.02	7.57	8.39	14.43	18.74	29.37	27.32	18.38	12.15 (10.77-13.53)
1992	0.91	4.91	9.92	12.05	16.40	17.23	33.84	33.23	31.74	14.11 (12.62-15.60)
1993	1.21	4.67	9.58	12.10	14.12	25.94	36.56	30.31	27.04	14.74 (13.22-16.27)
1994	0.91	5.95	11.09	11.09	11.29	20.13	31.12	40.51	20.89	13.90 (12.42-15.37)
1995	0.00	5.33	8.80	10.41	19.96	22.53	30.72	38.69	31.40	14.84 (13.31-16.36)
1996	0.93	6.88	7.72	12.19	17.70	23.03	30.81	33.95	38.59	15.00 (13.46-16.53)
1997	1.25	5.27	10.69	10.83	19.69	25.57	29.88	38.07	38.68	15.89 (14.31-17.47)
1998	0.95	6.12	9.96	11.86	17.33	23.55	39.53	32.07	24.14	15.79 (14.21-17.36)
1999	2.25	3.37	12.44	12.15	16.33	26.99	33.39	40.43	41.08	16.66 (15.04-

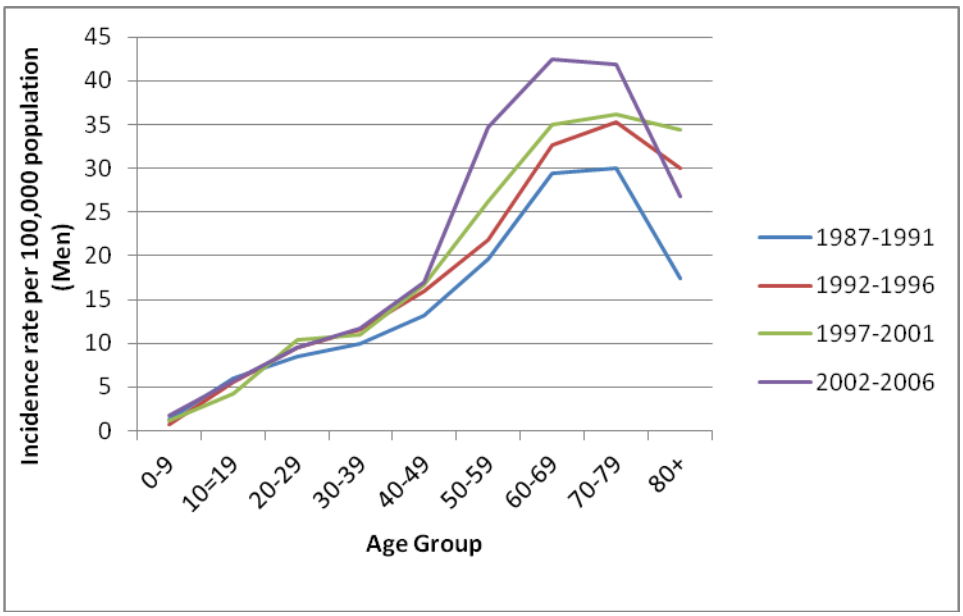
										18.28)
2000	0.33	3.06	7.64	10.72	16.45	30.71	34.93	37.95	40.40	16.12 (14.52-17.71)
2001	0.67	3.06	10.94	8.97	13.64	24.06	37.47	31.96	28.32	14.75 (13.23-16.28)
2002	1.38	7.92	8.13	13.16	17.33	33.51	34.80	39.99	23.93	17.81 (16.13-19.48)
2003	2.10	5.16	9.15	12.32	18.71	34.71	48.58	40.86	28.08	19.51 (17.76-21.27)
2004	0.71	6.04	12.64	9.52	13.79	29.09	44.79	46.72	31.70	18.23 (16.54-19.92)
2005	1.77	4.56	10.11	12.41	16.78	37.22	43.17	39.39	26.35	19.10 (17.37-20.82)
2006	2.49	4.60	7.63	11.21	18.25	38.61	40.61	42.57	24.00	19.07 (17.35-20.80)

Table 3.5a - Age specific annual incidence rate of RRD in men between 1987-2006

FEMALE	Incidence per 100,000 Age Group									
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80+	All Ages (95% CI)
Year										
1987	0.96	2.78	4.83	6.90	5.17	7.90	24.48	27.25	14.91	9.08 (7.93-0.23)
1988	0.64	2.61	4.61	7.20	7.93	16.54	22.08	18.02	14.48	9.30 (8.14-10.47)
1989	0.32	2.10	4.37	4.27	8.41	18.29	22.49	24.73	13.25	9.56 (8.38-10.74)
1990	0.63	1.23	3.40	6.98	7.98	19.07	24.68	31.03	14.73	10.54 (9.30-11.78)
1991	0.00	1.26	3.41	8.48	6.04	14.35	28.60	24.12	17.66	9.93 (8.73-11.13)
1992	0.32	2.24	4.44	6.43	9.57	17.19	24.47	23.04	11.85	9.92 (8.72-11.12)
1993	0.95	1.94	4.26	7.33	7.71	17.06	32.76	27.34	21.84	11.50 (10.21-12.79)
1994	0.32	0.97	4.10	5.37	10.56	20.71	30.75	32.63	11.57	11.48 (10.19-12.77)
1995	0.32	0.97	6.05	7.02	8.10	19.63	30.13	28.41	18.23	11.51 (10.22-12.80)
1996	0.65	1.29	4.61	8.43	6.28	19.54	22.81	24.00	15.95	10.17 (8.95-11.38)
1997	0.65	1.92	7.33	4.18	6.62	16.88	27.71	33.53	15.34	11.02 (9.75-12.28)
1998	0.33	0.63	4.98	4.65	6.59	16.81	32.14	24.37	24.86	10.80 (9.55-12.06)
1999	0.67	2.85	6.03	5.38	8.79	12.70	26.17	27.72	19.70	10.62 (9.38-11.87)
2000	1.03	1.59	5.84	5.89	8.66	15.96	26.58	25.31	18.30	10.76

										(9.50-12.01)
2001	0.35	1.59	5.32	3.94	5.75	17.53	33.82	35.59	19.27	11.78 (10.47-13.10)
2002	0.36	1.91	6.42	6.24	8.58	16.74	33.88	25.92	24.05	12.12 (10.79-13.46)
2003	0.37	1.91	5.53	7.13	8.64	19.29	29.06	26.41	15.22	11.63 (10.32-12.93)
2004	0.37	1.27	5.84	7.03	7.41	18.14	27.98	33.84	17.92	11.93 (10.61-13.25)
2005	0.37	1.91	3.83	7.23	6.76	18.18	30.07	30.05	12.08	11.33 (10.05-12.62)
2006	0.75	1.61	5.90	7.16	8.64	17.05	28.80	29.57	15.39	11.75 (10.44-13.05)

Table 3.5b – Age specific annual incidence rate of RRD in women between 1987-2006



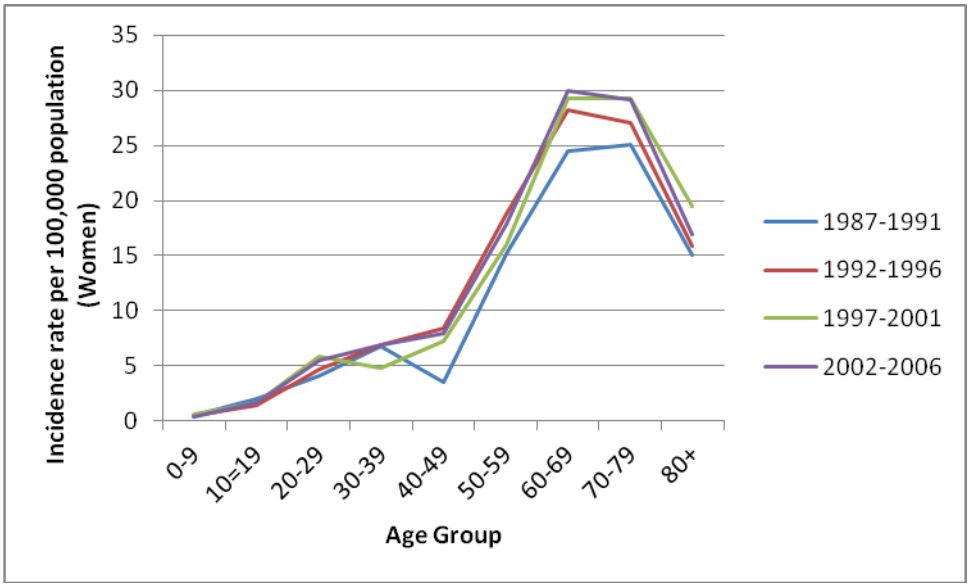


Figure 3.5a+b – Age specific annual admission rates of RRD in men and women over four time periods. In men, over the four time periods, a progressively higher incidence was noted in men of younger age. This pattern was absent in women.

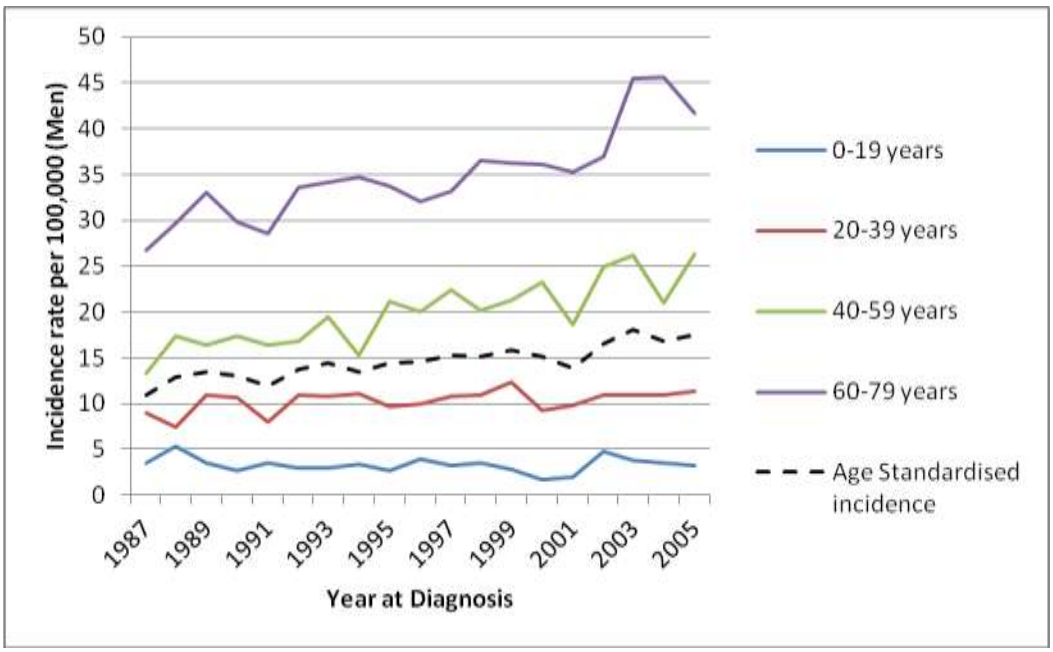


Figure 3.6a – Age sepcific and standardised incidence of RRD in men. A signficant rising trend was found in all age groups combined(χ^2 trend=154.96, p-value < 0.001) and in age groups 40-59years (χ^2 trend=71.43, p-value < 0.001) and 60-79years (χ^2 trend=42.22, p-value < 0.001).

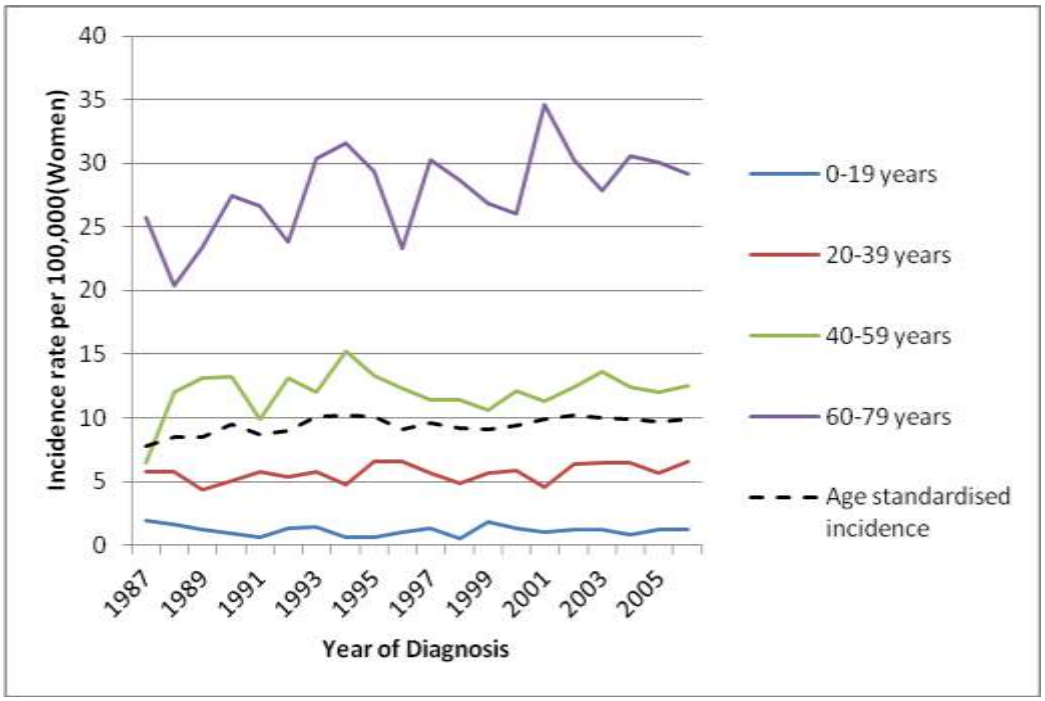


Figure 3.6b - Age sepcific and standardised incidence of RRD in women. A signficant rising trend was found in all age groups combined (χ^2 trend=27.84, p-value < 0.001) and in age group 60-79years only (χ^2 trend=12.35, p-value < 0.001).

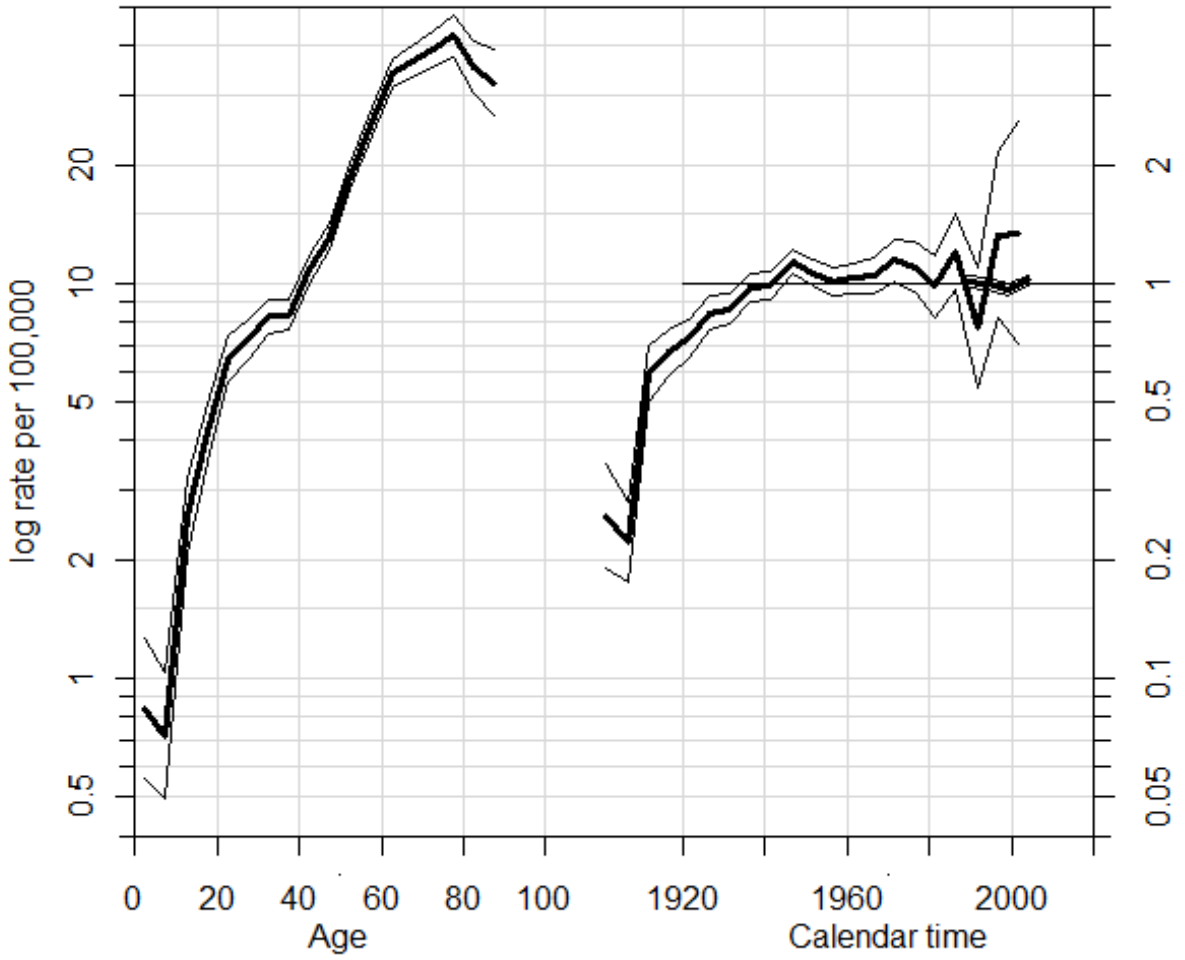


Figure 3.7 - Age-period-cohort plot of parameter estimates and associated 95% confidence intervals of RRD incidence in Scotland. This figure highlights the increasing incidence of RRD with age, demonstrating a peak in the 6th decade and a smaller secondary peak in the 3rd decade. No significant period effects were noted. There were no significant effects in recent birth cohorts.

3.4 Discussion

The CSR in Scotland has climbed linearly and increased 4 fold since 1986. This is not surprising, as recent cataract surgery initiatives in the UK have increased surgical capacity and estimates from other developed countries have indicated a similar trend; in the United States, the estimated prevalence of pseudophakia or aphakia is 5.1% of those over forty (6.1million), and this is expected to rise (to 9.5million) by 2020. (Ninn-Pedersen & Bauer 1996; Dua *et al.* 2009; Congdon N *et al.* 2004)This rise in the CSR has prompted questions about the overuse of cataract surgery, and as uncomplicated cataract surgery remains a risk factor for RRD development(Austin *et al.* 1990; Lois & Wong 2003), this rise in CSR may have important implications in the increase in RRD incidence noted in Scotland.

The overall crude incidence rate of RRD worldwide from studies of adequate methodology has been reported between 6.3 and 17.9 per 100,000 of population(Mitry *et al.* 2009a; Algvere *et al.* 1999; Li 2003; Haimann *et al.* 1982a; Limeira-Soares *et al.* 2007; Rowe *et al.* 1999; Laatikainen *et al.* 1985; Polkinghorne & Craig 2004b; Zou *et al.* 2002; Wilkes *et al.* 1982), however, many studies do not report the standardised incidence rates and have not examined incidence trends over a long time period, making it difficult to determine if the rise in incidence noted in Scotland is also present in other populations. Based on HES data, the estimated age standardised incidence rate of RRD in Scotland was 13.61 per 100,000 of population in 2006.

Over 20 years in Scotland, there is an average annual percent increase in RRD incidence of 1.9%.

A proportion of this increase in incidence is attributable to temporal changes in the Scottish population and the rising proportion of elderly individuals. However, the statistically significant difference noted between the expected and observed incidence rates in 2006 suggests that there are other factors which may have influenced the trend observed. The proportion of myopia, previous cataract surgery, ocular trauma and the socio-economic status of a study population can all affect RRD incidence.(Saidkasimova *et al.* 2009; Austin *et al.* 1990) Temporal changes in the prevalence of these risk factors in Scotland are likely to also contribute to the observed trend in incidence. Furthermore, the advances in vitreo-retinal surgery and the expansion of specialist vitreo-retinal services in Scotland between 1987 and 2006, may have increased the number of operable cases and subsequently contributed to the rise in disease incidence noted.

The incidence of RRD in both men and women peaks in the 6th decade with a secondary peak in the 3rd decade, which is widely supported by previous population based estimates.(Algvere *et al.* 1999; Li 2003; Haimann *et al.* 1982a; Limeira-Soares *et al.* 2007; Rowe *et al.* 1999; Laatikainen *et al.* 1985; Polkinghorne & Craig 2004b; Zou *et al.* 2002; Wilkes *et al.* 1982) Examining sex-specific RRD rates over 20 years, I found a higher incidence in males in virtually all age groups with an age standardised male:female incidence ratio varying between 1.31:1 to 1.82:1. The higher incidence in men has been reported in previous studies with male:female incidence ratios varying between 1.3:1 to 2.3:1.(Mowatt *et al.* 2003; Ivanisevic *et al.* 2000; Rosman *et al.* 2001; Polkinghorne & Craig 2004b; Limeira-Soares *et al.*

2007) This gender imbalance in RRD incidence in our study cannot be explained by the underlying gender distribution of the Scottish population and a higher rate of traumatic RRD in men or an inherent increased risk in males may be contributory.(Wong *et al.* 1999; Sheu *et al.* 2007)

There is also a temporal trend towards an earlier age of onset in males. There is a significant increase in RRD incidence in males of younger age groups (40-59 years) across the 20 year study period when compared to females of similar age. The reason for this is uncertain, but may be due to differences in the levels of myopia between genders or due to lifestyle differences, where males may under-report 'minor' ocular trauma. Previous studies have indicated that in younger myopic populations, males tend to predominate(Hirsch & Ditmars 1969; Hirsch M.J. 1953; Bourne *et al.* 2004; Dandona *et al.* 2002; Sawada A *et al.* 2008; Attebo *et al.* 1999), and in certain populations the influence of myopia, axial length and cataract surgery in males confers a higher risk for RRD development when compared with females.(Sheu *et al.* 2007)

An accurate estimate of disease incidence is an important first step in assessing the related healthcare burden. These results from national hospital data in Scotland over a 20-year period indicate a higher age standardised incidence in males and an increasing incidence of primary RRD in both sexes. The increase in RRD incidence may be partly attributed to the aging population in Scotland over the study period but other contributing factors may also exist. The increase in overall RRD incidence during the study period was more notable in males with a trend towards an earlier age of onset. Despite accounting for temporal changes the Scottish population, the reason for this imbalanced gender distribution is unclear. With the changes in population structure and a longer living population, it is likely that RRD will continue to add to the burden on ophthalmic services in Scotland.

CHAPTER 4 Genetics of RRD, Myopia and associated vitreoretinopathy

4.1 The Heritability of RRD

4.1.1 Evidence for the heritability of RRD

There is now significant evidence that ocular and retinal disorders related to RRD carry an important genetic component. Risk factors strongly associated with development of RRD such as myopia and lattice degeneration aggregate significantly in families. An eye with a spherical equivalent refractive error of between -1 to -3 dioptres(D) has a fourfold increased lifetime risk of RRD compared to a non myopic eye, increasing to tenfold with a refractive error of >-3D. (The Eye Disease Case-Control Study Group 1993) Multiple familial studies support a high genetic effect for myopia and heritability estimates calculated from twin studies are as high as 0.8.(Teikari *et al.* 1991; Teikari *et al.* 1988a) Lattice degeneration, implicated in up to two thirds of cases is the most prevalent vitreoretinal degeneration predisposing to RRD.(Straatsma BR *et al.* 1974) Family pedigrees have demonstrated autosomal dominant and recessive modes of inheritance, with a three-fold higher prevalence of lattice degeneration in first degree relatives.(Everett 1968)

A positive (first degree relative) family history in patients with RRD not associated with other ocular or non-ocular findings has been reported between 1 – 8.2% in large population studies.(Wilkes *et al.* 1982; Zou *et al.* 2002; CUENDET *et al.* 1975) A hereditary predisposition to rhegmatogenous retinal detachment was initially suggested over 40 years ago and recently an

autosomal dominant gene mutation with variable penetrance for RRD has been identified in a genetic linkage study. (FRANCOIS 1968; Francois J & Verbraeken H 1975; CUENDET *et al.* 1975)Inherited vitreoretinopathies associated with RRD such as Stickler syndrome, Wagner syndrome and familial exudative vitreoretinopathy(FEVR) have been well defined in their mode of inheritance and genetic heterogeneity(Snead & Yates 1999b), however the genetic risk of non-syndromic RRD in the general population is as yet, poorly characterised. A hereditary predisposition to rhegmatogenous retinal detachment was reported over 40 years ago and recently an autosomal dominant gene mutation with variable penetrance has been identified in a genetic linkage study. (FRANCOIS 1968)'

Most remarkably however, Go *et al* have demonstrated that familial occurrence of RRD is a risk factor for its' development, with a risk ratio of 2.6 for cumulative lifetime risk of RRD in relatives of subjects with RRD compared to relatives of those without RRD.(Go *et al.* 2005b) Despite controlling known risk factors (myopia, age and sex), siblings of subjects with RRD had a 3-fold increased frequency of RRD compared with siblings of non-affected subjects. Go *et al* have also reported autosomal dominant RRD in 2 families with minimal systemic features of Stickler syndrome to be associated with an Arg453Ter mutation in the COL2A1 gene suggesting a wide phenotypic heterogeneity associated with this condition.(Go *et al.* 2005a)These patients were significantly younger than the general population of patients who present with primary RRD. (Average age 36 in family A and 14 in family B), and it would appear that these detachments are more difficult to treat and more likely to develop PVR.(Theelen T *et al.* 2004)

4.1.1.1 Retinal Dialysis and Giant Retinal Tears

Retinal dialysis is a distinct type of retinal break which can account for 1.8-10% of RRD.(Kinyoun J.L & Knobloch W.H 1984; Vaiser A. & Jost B.F. 1992) Some may be associated with ocular trauma but others may be idiopathic or familial. A study of 30 pedigrees of patients with retinal dialysis revealed a high concentration of cases in sibships examined (33%). The mode of inheritance could not be postulated for all cases, though the vast majority of cases had normal parents suggesting a possible recessive inheritance.(Verdaguer *et al.* 1975) The authors observed bilateral rate of 54% percent; none of whom were significantly myopic, suggesting a genetic origin in some of the patients.

Some authors believe that virtually all retinal dialyses occur secondary to trauma.(Ross 1981; Ross 1991) Kinyoun et al failed to show a significant association with genetic causation, a series of over 30 retinal dialyses failed to show a familial tendancy.(Kinyoun J.L & Knobloch W.H 1984) Hagler's series of over 500 consecutive dialysis cases showed that less than 2% had a family history of dialysis or bilateral dialyses inferring that a genetic cause was unlikely.(Hagler 1980)

However, the high rate of reported bilateral dialysis(Verdaguer *et al.* 1975; Hilton & Richards 1970) and numerous twin studies suggest a genetic predisposition in at least some cases.(Vaiser A. & Jost B.F. 1992) Recently, atrophic retinal holes have been reported in non traumatic phakic myopic monozygotic twins.(Chan *et al.* 2006)

Idiopathic giant retinal tears are uncommon and tears may be traumatic or idiopathic. However there is a high rate of bilateral involvement, a tendency to occur preferentially in males and in high

myopes.(Freeman 1978; Kanski 1975) Similarly, recent twin reports have raised the possibility of genetic influences in the pathogenesis of giant retinal tears.(Kumar *et al.* 2006; Chaudhry N.A. *et al.* 1999)

4.1.2 Myopia and Rhegmatogenous Retinal Detachment

Myopia is the most common eye disorder in the world.(Tang *et al.* 2008) It is an error of refraction primarily caused by increased axial length, but may also be caused by corneal or lenticular changes. (Angle & Wissmann 1980; Young *et al.* 1998) Axial length is the principal determinant of myopia and has been shown to have a heritability of 0.4-0.94.(Lyhne *et al.* 2001; Biino *et al.* 2005; Klein *et al.* 2009) The distribution of myopia has been found to be patterned in its appearance in different races and ethnic groups.(Angle & Wissmann 1980)

Higher levels of myopia, usually defined as an axial eye length of >26 mm or a refractive error of >-6.00 diopters is often called 'pathologic' myopia, because of the predisposition to develop further eye disorders such as retinal detachment, macular degeneration, cataract, or glaucoma. (Xu *et al.* 2007; Curtin BJ 1985; Curtin BJ & Karlin DB 1971) High myopia is the fourth most common cause of irreversible blindness because of associated complications. (Katz *et al.* 1997; Curtin BJ 1970) The prevalence of high myopia varies worldwide. High myopia (>-5D) has a prevalence if 4.5%, 4.6% and 2.8% in over 40 year old adults in the United States, Western Europe and Australia respectively.(Kempen *et al.* 2004) In Mexico high myopia was seen in 1.4% of the population with a statistically significant higher prevalence in females. The prevalence of high myopia in Asians is higher than in Caucasians being 8.2% in Japan, 9.1% in Singaporean Chinese and 21% in

Taiwan students.(Sawada A *et al.* 2008) Highly myopic patients can represent up to 33% of the myopic population. (Curtin 1979)

The aetiology of non-syndromic myopia is influenced by the broad clinical spectrum of this condition, genetic heterogeneity, and potential environmental modulating factors such as close work (which may account for 12% of phenotypic variation).(Nallasamy *et al.* 2007) The influence of genetic heredity on high myopia is a significant factor in its etiology.(Zhang *et al.* 2007; Naiglin *et al.* 2002)'(Ashton 1985; Yap *et al.* 1993; Mutti *et al.* 2002) Family aggregation studies demonstrate a myopia prevalence of 7.3% in children of emmetropic parents, increasing to 45% if both parents are myopic. Similarly, the risk of myopia in children with highly myopic parents is 4.6-15 fold higher than with non myopic parents and 1.5-3 fold higher for moderate myopia, suggesting a strong genetic basis for high myopia and a possible genetic basis for low or moderate myopia. Twin studies have also reported convincing evidence, with dizygotic heritability (the proportion of total phenotypic variance attributed to genetic variation) calculated at 0.5 and monozygotic heritability of the condition are as high as 0.8 to 1.(Hammond *et al.* 2001; Teikari *et al.* 1988b) The λs for myopia (the increase in risk to siblings of a person with the disease compared to population prevalence) has been estimated to be 4.9 to 19.8 for sibs for high myopia and 1.3 to 3 for sibs for low or common myopia, suggesting a definite genetic basis for high myopia and a strong genetic basis for low myopia.(Guggenheim *et al.* 2000; Farbrother *et al.* 2004) Previous studies have demonstrated an autosomal dominant, autosomal recessive(SORSBY & Benjamin 1973) and X-linked recessive mode of inheritance.

4.1.2.1 High Myopia – Genes Identified

Much of the current information on human myopia molecular genetics can be drawn from familial studies of high myopia and more recently, genome wide association studies. To date there are at least 16 identified loci associated with high myopia established through familial linkage analysis. These are summarised in table 4.1**.** An X-linked recessive form of myopia to chromosome Xq28 (MYP1) has been shown to be associated with cone dysfunction.(Schwartz *et al.* 1990) More recently X linked recessive myopia has been mapped to Xq23-25(MYP13)(Zhang *et al.* 2006)in Chinese families, and further studies implicate hepatocyte growth factor and myocilin as putative candidate genes.(Yanovitch *et al.* 2009; Vatavuk *et al.* 2009) A recent genome wide association study associated and replicated a locus on chromosome 11q24.1 with high myopia in a Japanese population. (Nakanishi *et al.* 2009), and several European consortia have identified and replicated susceptibility loci on chromosome 15.(Verhoeven *et al.* 2012) Different genetic loci have been mapped and replicated to different extents in population groups, but none of the responsible genes have as yet been identified.

Myopia	Genetic	Region of	LOD	Replication	OMIM	Myopia
MYP1[307]	Xq28	Bornholm,	4.8	Yes	310460	-6.75to-11.25
MYP2[301]	18p11.31	US Families	9.59	Yes	160700	-6 to -21
MYP3[281]	12q21-q23	US Family	3.85	No	603221	-6.25 to -15
MYP4[292]	7q36	French	2.81	No	608367	-13.05 (avg)
MYP5[303]	17q21-q22	Canadian	3.17	No	608474	-5.5 to -50
MYP6[311]	22q12	Ashkenazi	3.08	Yes	608908	≥-1.0
MYP7[312]	11p13	British Twins	6.1	No	609256	-12.12to 7.25
MYP8[312]	3q26	British Twins	3.7	Yes	609257	-12.12to 7.25
MYP9[312]	4q12	British Twins	3.3	No	609258	-12.12to 7.25
MYP10[312]	8p23	British Twins	4.1	No	609259	-12.12to 7.25
MYP11[306]	4q22-q27	Chinese	3.1	No	609994	-5 to -20
MYP12[304]	2q37	US Family	5.67	Yes	609995	-7.25 to -27
MYP13[308]	Xq23-q25	Chinese	2.75	No	300613	-6 to -20
MYP14[313]	1p36	Ashkenazi	9.5	Yes	610320	QTL
MYP15[290]	10q21.1	Hutterite	3.22	No	612717	-7.04 (avg)
MYP16[314]	5p15.33	Chinese	4.81	No	612554	NK

Table 4.1 – The validated Online Mendelian Inheritance in Man (OMIM) genetic loci associated with myopia. (NK - No known)

4.1.3 Heritability of Lattice Degeneration

Lattice degeneration is a prevalent clinical feature associated with myopia in a significant proportion of rhegmatogenous retinal detachments.(Straatsma BR *et al.* 1974; Folk J.C. *et al.* 1989) Family studies have proposed a hereditary factor implicated in the development of lattice degeneration.(Byer N.E 1979) Everett described a family of 88 people without myopia in whom 22% showed lattice. This pedigree reported an autosomal dominant mode of inheritance with 80% penetrance.(Everett 1968) Other reports on large families have also implicated autosomal dominant transmission with incomplete penetrance.(Delaney W.V *et al.* 1963) Lewkonia et al have reported the hereditary pattern to be autosomal recessive pseudodominant which they consider explains the high prevalence seen in the general population.(Lewkonia I. *et al.* 1973) Murakami et al performed an in depth

examination of 100 consecutive patients with lattice degeneration without retinal detachment. The prevalence of lattice degeneration in first degree relatives was three times higher in this cohort than in the general population. Polygenic inheritance was proposed as no specific pattern of inheritance could be delineated.(Murakami F. & Ohba N. 1982) Most recently however, a GWAS from Japan has reported compelling evidence of an association between lattice degeneration and COL4A4, a component which has been localised to the vitreoretinal interface suggesting the role of basement membrane formation in the pathogenesis of lattice degeneration.(Meguro *et al.* 2012)

4.2 Genetics of Common Hereditary Vitreo-retinopathy

4.2.1 Stickler Syndrome - Clinical Features

Stickler syndrome is an autosomal dominant disorder of connective tissue with characteristic ocular, facial, auditory and articular manifestations.(STICKLER *et al.* 1965) It is a common connective tissue dysplasia and the commonest cause of inherited retinal detachment.(Richards *et al.* 1996; Scott J.D 1980) It is a genetically and phenotypically heterogenous disorder with the majority of families having mutations in the COL2A1 gene, coding for type II collagen, a major constituent of vitreous and cartilage. (Ahmad *et al.* 1995; Donoso *et al.* 2003b; Fryer *et al.* 1990) There are no agreed diagnostic criteria for Stickler syndrome, but Snead and Yates' widely accepted criteria are:(Snead & Yates 1999a)

Congenital vitreous anomaly with any three of the following:

- Myopia with onset before 6 years
- Rhegmatogenous retinal detachment or paravascular pigmented lattice degeneration
- Joint hypermobility with abnormal Beighton score with or without radiological evidence of degeneration
- Audiometric confirmation of sensorineural hearing loss
- Midline cleft palate

Vision and Refractive Error

The large majority of patients with Stickler syndrome are myopic (90%)(Donoso *et al.* 2003b). The myopia of Stickler syndrome is usually congenital of a high degree and is non progressive. (Snead & Yates 1999a)

Anterior Segment

Developmental abnormalities of the anterior chamber have been described and may predispose to glaucoma.(Nielsen 1981)

Vitreous

Vitreous abnormalities are described as pathognomonic of Stickler syndrome. Two clear phenotypes have been defined: (Snead *et al.* 1994; Snead & Yates 1999a; Snead *et al.* 1996a; Snead 1996b)

Type 1 Anomaly (Membranous) - Vestigial gel remnant which occupies the anterior vitreous cavity and extends in a thin sheet over the pars plana anteriorly and may extend post equatorially. It is described as an optically empty vitreous in the retrolental space which is bordered by a distinct folded membrane.

Type 2 Anomaly (Fibrillar) – Sparse and irregularly thickened bundles of variable diameter fibres scattered throughout the vitreous cavity. These may be associated with localised or complete separation of the posterior hyaloid membrane.

Retina

A characteristic perivascular pigmentary degeneration is seen in this condition. These lesions are similar to lattice degeneration, however they are broad, found post equatorially with an ill defined margin. Rhegmatogenous retinal detachment including giant retinal tears occurs in more than 60% of subjects over their lifetime.(Snead 1996a; Donoso *et al.* 2003b)

Systemic manifestations:

Facial manifestations

Facial abnormality is present in up to 84% of cases.(Donoso *et al.* 2003a) The classically described features are a flattened mid face, depressed nasal bridge, reduced nasal protuberance, anteverted nares and micrognathia. These are usually most evident at a young age. One quarter of affected patients have cleft abnormalities, which vary in their severity from mild (bifid uvula) to severe (Pierre – Robin). (Temple 1989)

Deafness

This may be conductive secondary to chronic otitis media from cleft abnormalities or more commonly it may be sensorineural in origin. The pathogenesis of the sensorineural hearing loss is unknown, but is present in up to 40-70% (Snead & Yates 1999a; Donoso *et al.* 2003b) of subjects. It is usually a subtle loss to high tones, which may not be noticed by the patient. It is thought to be congenital in origin and does not progress throughout life.(Szymko-Bennett *et al.* 2001) It has also been observed that the hearing loss varies depending on the type of Stickler syndrome, being mildest with type 1.(Szymko-Bennett *et al.* 2001)

Joint Hypermobility

This is present in many young subjects with Stickler syndrome and reduces with growth. Most adult subjects develop a subsequent degenerative arthropathy by the third or fourth decade.(Rai *et al.* 1994)

Genes Identified

The first causative mutations in Stickler subjects were identified in the COL2A1 gene which was also found in the original Stickler pedigree. (Martin *et al.* 1999) Further research has provided some genetic evidence to sub-classify Stickler according to the phenotypic appearance. Up to 75% of patients with type 1 vitreous Stickler syndrome show linkage to COL2A1 on chromosome 12q13. (Snead & Yates 1999a)

More recently, mutations in the gene encoding the alpha one chain of type XI collagen (COL11A1) on chromosome 1p21 have been found in families with type II fibrillar vitreous anomaly giving rise to what is sometimes referred to as type II Stickler syndrome.(Richards *et al.* 1996; Martin *et al.* 1999; Fryer *et al.* 1990; Bonaventure *et al.* 1992) Linkage to COL2A1 in patients with type II vitreous anomaly has been excluded. (Snead *et al.* 1994; Snead *et al.* 1996b) A predominantly systemic form of Stickler syndrome has also been reported with mutations in the COL11A2 gene which is not expressed in ocular tissue.(Brunner *et al.* 1994; Vikkula *et al.* 1995)

Collagen II and XI are in a family of fibrillar collagens which are found to predominate in the vitreous, cartilage and nucleus pulposus. (Snead & Yates 1999a) Collagen II accounts for the majority of vitreous collagen and is responsible for its gel structure.(Forrester *et al.* 1996) Type XI collagen regulates collagen fibril diameter and mutations in the coding gene are thought to result in thickened, irregular, beaded vitreous strands.(Blaschke UK *et al.* 2000; Richards *et al.* 2006; Poulson *et al.* 2004)

The COL2A1 mutations are usually stop mutations(Snead & Yates 1999a) and result in haploinsufficiency via nonsense mediated decay,(Richards *et al.* 2006) although entire COL2A1 allele deletions have been reported.(Van Der

Hout *et al.* 2002)The phenotypic appearance of the vitreous has been correlated to a large part with the genetic mutation, with type 1 being linked to COL2A1 and type 2 showing linkage to COL11A1. However there have been cases of type I vitreous phenotype with a COL11A1 mutation(Parentin *et al.* 2001)A third locus is suspected, as in several Stickler pedigrees linkage to COL2A1 and COL11A1 has been excluded. (Wilkin *et al.* 1998; Snead & Yates 1999a; Snead 1996b)

4.2.3 Wagner Syndrome - Clinical Features

Wagner first described a vitreo-retinal disorder with an autosomal dominant mode of inheritance in a Swiss pedigree.(Wagner H 1938) Patients were characterised as having mild myopia, vitreoretinal abnormalities and early onset cataract without extra-ocular manifestations. The disease may be evident in childhood and has a progressive clinical course. (Graemger RA *et al.* 1995) Additional findings reported include peripheral perivascular hyperpigmented regions of the retina, vascular sheathing, and visual field abnormalities. This is in contrast with Stickler syndrome where retinal function is normal, and visual loss is non-progressive. (Meredith SP *et al.* 2007)

Vision and Refractive Error

Visual acuity progressively declines with age and by 50 years only 25% of patients have vision better than 20/100 (6/36). Studies indicate this to be related to the extent of chorioretinal degeneration illustrated by electro-retinogram (ERG) worsening. (Zech *et al.* 1999; Graemger RA *et al.* 1995) Although documented refractive errors indicate a mild to moderate myopia (-3D or less), between 25-32% of subjects observed have myopia >-6D.

Anterior Segment

Visually significant dot like cortical cataract and nuclear sclerosis is present in nearly all patients by the age of 33.(Zech *et al.* 1999)

Vitreous

The vitreous cavity is characteristically described as being optically empty apart from being traversed by membranes and strands or veils. These progressive alterations may occur early and are reported in the pre-adolescent age group of affected individuals. Clinically, one of the prominent features has been described as the thickening and incomplete separation of the posterior hyaloid membrane, which occurs in a circular avascular pre-retinal membrane sometimes referred to as a veil or rope.(Meredith SP *et al.* 2007; Graemger RA *et al.* 1995; Brown MB *et al.* 1995)

Retina

Progressive pigmentary changes, punched out chorioretinal atrophy, loss of RPE and lattice degeneration are significant retinal features in Wagner syndrome. Whitish equatorial vitreous membranes, perivascular pigmentary clumping and and macular dragging have been observed.(Maumenee 1979; Michels RG *et al.* 1990) In Wagners' original pedigree 54% of eyes affected exhibited an abnormal pattern of retinal vessels. (''dragged vessels''). Pseudostrabismus and ectopic fovea have also been described. (Meredith SP *et al.* 2007; Miyamoto T *et al.* 2005)

Retinal breaks have been reported in up to 75% of patients, 47% of those having an associated retinal detachment (combined tractional and rhegmatogenous). (Hirose *et al.* 1973) Follow up of the original Wagner pedigree suggests a much more infrequent incidence of RRD but tractional retinal detachment was seen in 35% of cases with an older average age of 49 years.(Graemger RA *et al.* 1995) In contrast, RRD occurs much earlier

with an average age as young as 16 years.(Zech *et al.* 1999) The prevailing sense is that the risk of RRD in Wagner syndrome is low compared with other vitreoretinopathies such as Stickler syndrome and erosive vitreoretinopathy.(Brown *et al.* 1994; STICKLER *et al.* 1965)

Retinal Function

Electrophysiological abnormalities such as elevated rod and cone thresholds on dark adaptation and subnormal b-wave amplitudes on the electroretinogram which correlate with the severity of chorioretinal atrophy also feature as part of this syndrome.(Graemger RA *et al.* 1995)

Genes Identified

Brown et al first showed Wagner disease to be linked to chromosome 5q13-14.(Brown MB *et al.* 1995) Further studies showed the localisation of the gene to a 2.5cM region on chromosome 5q14.3(Perveen R *et al.* 1999; Zech *et al.* 1999) which has more recently been confirmed in a Japanese family. (Miyamoto T *et al.* 2005)

Analysis of a candidate gene CSPG2 found a single base pair change in one family, which was absent in 100 controls.(Perveen R *et al.* 1999) However, no mutation was found in 17 other families. Identification of the genetic defect in the original Wagner pedigree revealed a heterozygous substitution which cosegregated with the disease phenotype.(Kloeckener-Gruissem *et al.* 2006)

4.2.5 Erosive Vitreoretinopathy - Clinical Features

First described in 1993 this is hereditary vitreo-retinopathy is exhibited symptomatically by nycthalopia and visual field loss and clinically, by a

syneritic vitreous, RPE thinning and atrophy as well as RRD. In the original series, combined tractional and rhegmatogenous retinal detachment was found in 42% of the pedigree with the vast majority occurring under the age of ten years.(Brown *et al.* 1994) As with other vitreoretinopathies, considerable variation in expression exists within pedigrees.

Vision and Refractive Error

The visual prognosis is poor with 20% of eyes having an acuity of PL or worse from retinal detachment, as well as this a further 20% have a visual acuity of 20/200 or worse from RPE and/or photoreceptor damage. Refractive errors have been seen to vary between emmetropia and -10diopters, but most remain in the myopic range. Visual field testing in these patients reveals progressive constriction of visual fields as well as peripheral or central scotoma corresponding to the erosive lesions.

Anterior Segment

Nuclear sclerotic cataract is common in affected eyes usually necessitating extraction by the age of 40.

Vitreous

Vitreous syneresis is seen in all affected patients with pronounced vitreous sheet, veils and ‘’ropes.’’ Vitreous traction is also common at the border of the erosive lesion and normal appearing RPE.

Retina

Progressive RPE changes allowing visualisation of the underlying choroid are manifest in all patients with this condition. Progressive thinning or erosion of the RPE leading to geographic atrophy with scalloped edges is often seen around the equatorial areas. In the original series 11 0f 15 patients had a rhegmatogenous retinal detachment, 5 of those suffering bilateral RRDs. Nearly all of the detachments occurred in the age group under 10. Dragged

disc vessels, an ectopic macula and a positive angle kappa (strabismus) have also been noted. (Brown *et al.* 1994)

Retinal Function

Striking ERG abnormalities feature in this condition, which resemble those seen in retinitis pigmentosa. Both rod and cone photoreceptor dysfunction is evident. The light adapted 30Hz flicker was abnormally prolonged in cases of rod and cone dysfunction. Oscillatory potentials were minimally developed. No systemic manifestations are associated.

Genes Identified

The critical region associated with erosive vitreoretinopathy was found to overlap that with Wagner disease to a 2cM region on chromosome 5q14.3, giving rise to the concept that both conditions are allelic.(Brown MB *et al.* 1995)

4.2.7 Snowflake Vitreo-retinal Degeneration - Clinical Features

This autosomal dominant hereditary vitreoretinopathy was first described by Hirose et al in 1974. It was characterised by early onset visually significant cataract, fibrillar degeneration of the vitreous, and crystal-like retinal deposits resembling snowflakes. No systemic features were evident.(Hirose *et al.* 1974)

Vision and Refractive Error

In the absence of other ocular disease, a good visual acuity was maintained in the snowflake pedigree. The refractive error in these patients varied from high hyperopia to myopia, with the majority being in the myopic range. 21% of patients had high myopia of >-6 diopters.

Anterior Segment

Cataract was seen in all patients over 32 years. The type of cataract was not uniform. Anterior segment corneal guttata has been reported in the follow up study of the original pedigree.

Vitreous

The vitreous is characterised by fine swirling strands and liquefaction in affected individuals. This severe fibrillar degeneration was sometimes associated with localized thickening of the cortical vitreous over degenerated areas of the retina. These changes are seen in all snowflake subjects, and were reported as early as 12 years of age suggesting they may be congenital changes.(Lee M.M *et al.* 2003; Robertson *et al.* 1982)

Retina

Fundal appearance in the original series has been described as being a progressive degeneration passing through four stages:(Hirose *et al.* 1974)

Stage 1 - 'White with pressure'lesions around the peripheral fundus with minimal vitreous degeneration

Stage 2 - Crystalline snowflake deposits which are found in the superficial layers of the retina and extend to the ora serrata, they may also extend radially along retinal vessels

Stage 3 - Sheathing of retinal vessels and increased peripheral pigmentation

Stage 4 - Increased retinal pigmentation and the disappearance of peripheral retinal vessels became apparent in patients aged over 58. The peripheral retina appears markedly atrophic and severe vitreous degeneration is evident.

Radial perivascular degeneration seen frequently in Stickler subjects were noted in 33% of snowflake patients. Minute yellow-white crystals like deposits seen in the peripheral retina appear to be a unique finding in these subjects. Lattice degeneration was absent. RRD was seen in 21% of

subjects at a mean age of 60. Tractional RD and retinal vessel abnormalities typical of Wagner disease were not seen. (Lee M.M *et al.* 2003)

Retinal Function

Elevated dark adaptation and reduced scotopic B wave with dim light are electrophysiological abnormalities evident in most snowflake subjects.(Hirose *et al.* 1974)

Genes Identified

A genome-wide scan of a pedigree with snowflake vitreoretinal degeneration showed linkage to a 20 Mb genetic locus on chromosome 2q36 flanked by D2S2158 and D2S2202.(Jiao X *et al.* 2004) Most recently, it has been demonstrated that within this region a heterozygous mutation in KCNJ13 (coding a potassium rectifier channel) can cause snowflake vitreoretinal degeneration. This gene encodes Kir7.1 which has been localised to the RPE and inner-limiting membrane of human retina. (Hejtmancik *et al.* 2008)

4.2.9 Familial Exudative Vitreoretinopathy - Clinical Features

Familial Exudative Vitreoretinopathy (FEVR) is an autosomal dominant nherited disorder of retinal vessel development that affects both eyes.(Benson 1995a) First reports (Criswick and Schepens) indicated that clinical features can be asymmetrical and highly variable even within the same family.(Criswick & Schepens 1969) The primary pathological process appears to be premature cessation of retinal angiogenesis leading to incomplete vascularisation of the peripheral retina, which may be evident on fundal fluorescein angiography (FFA) in asymptomatic individuals.(van Nouhuys 1991; Ober *et al.* 1980) The visual morbidity in these individuals develops from secondary complications such as peripheral

neovascularisation, falciform retinal folds, retinal traction, fibrovascular proliferation and exudative, tractional and rhegmatogenous retinal detachment.(Shukla *et al.* 2003)

Vision and Refractive Error

Visual acuity varies depending on age of onset and progression of secondary complications. Of note 50% of patients with FEVR will be assymptomatic.(Benson 1995a) It has been observed that patients with FEVR who have not had significant visual loss by the age of 20 tend to stabilise clinically and retain good visual acuity.(Gow & Oliver 1971; Ober *et al.* 1980; Feldman *et al.* 1983) In one series less than 10% of patients whose onset of symptoms occurred before their third birthday had a final visual acuity of 20/200.(Benson 1995a) There is no characteristic refractive error in FEVR with the majority being between +3 and -3 diopters.(van Nouhuys 1982)

Anterior Segment

Early onset posterior subcapsular cataract has been reported but it is not a characteristic feature.(Slusher & Hutton 1979; Boldrey *et al.* 1985)

Vitreous

Although originally postulated by Criswick and Schepens that the vitreous plays an important role in the development of the disease, it has subsequently been observed that vitreous may not play a primary role in the etiology, and up to 50% of subjects may have a normal appearing vitreous.(Miyakubo *et al.* 1982) Current clinical and pathological findings conclude that this is a disease of the small peripheral retinal vessels rather than a true vitreoretinopathy.(Laqua 1980; Criswick & Schepens 1969; Gow & Oliver 1971)

Retina

All affected individuals have an avascular peripheral retina, and in many cases this may be the only manifestation.(Benson 1995a) If this is the diagnostic criteria used, it is said that FEVR has 100% penetrance.(Benson 1995b)

Gow and Oliver established 3 clinical stages of FEVR:(Gow & Oliver 1971)

Stage 1 - Demonstrated vitreoretinal interface changes, vitreous bands and peripheral retinal degeneration - 'white-without pressure'

Stage 2 - Highlights a progression to neovascularisation, retinal exudation, elevated fibrovascular bands in the peripheral retina, localised retinal detachment, and dragging of the disc and macula.

Stage 3 - End stage disease with total retinal detachment, cataract formation, iris atrophy and neovascular glaucoma.

Significant vitreous adhesion and traction is a frequent cause of severe visual loss, being present in up to 41% of individuals.(Benson 1995a) The initial manifestation of this may be vitreomacular traction seen in up to 49% of affected eyes.(Miyakubo *et al.* 1982; Miyakubo *et al.* 1984) RRD is the most frequent form of retinal detachment present in 17 – 30% of eyes but is commoner in young subjects who also have a more rapid progression.(Miyakubo *et al.* 1982; Shukla *et al.* 2003; Tasman *et al.* 1981) Peripheral retinal neovascularisation is present in up to one fifth of eyes and is a poor prognostic sign.(Benson 1995a; Slusher & Hutton 1979)

Retinal Function

Electroretinogram, electro-oculogram, colour vision testing and visual fields are normal or mildly abnormal in affected individuals.(van Nouhuys 1982; Feldman *et al.* 1983; Ohkubo & Tanino 1987)No systemic manifestations have been reported.

Genes Identified

FEVR like most hereditary vitreoretinopathies is genetically heterogeneous. Autosomal dominant,(Laqua 1980; Gow & Oliver 1971) X linked (Plager *et al.* 1992; Shastry *et al.* 1997; Shastry *et al.* 1995) and autosomal recessive(de *et al.* 1998; Jiao *et al.* 2004) modes of inheritance have been described, with autosomal dominant being the most common.(Kondo *et al.* 2001b; Kondo *et al.* 2001a)

FEVR and Norrie disease share common molecular etiology and have highly overlapping ocular manifestations that are caused by alterations in the Wnt signalling network. Norrie disease is a severe X-linked recessive form of congenital blindness which is accompanied by mental retardation and deafness in one third of cases. (Berger *et al.* 1996) To date, mutations in at least four genes have been identified as responsible for autosomal dominant (FZD4, LRP5, and TSPAN12 genes) (Li *et al.* 1992) (Toomes *et al.* 2004) (Downey *et al.* 2001), autosomal recessive (LRP5)(Yang *et al.* 2012) or X-linked (NDP gene) (Fullwood *et al.* 1993) forms of FEVR. Mutations in LRP5 are also known to cause bone abnormalities.(Nikopoulos *et al.* 2010) The encoded proteins of these four genes are involved in the Wnt signalling pathway, which regulates retinal vascular development. Retinal vascular angiogenesis is depende nt on TSPAN12, FZD4 and LRP5 to initiate the B-catenin signalling cascade upon NDP binding, which is thought to play a critical role in ocular development and formation of the hyaloid vascular system during organogenesis.(Nikopoulos *et al.* 2010) FZD4, LRP5, and NDP are relatively small genes making sequence analysis of these genes cost effective for molecular testing.(Nikopoulos *et al.* 2010; Boonstra *et al.* 2009)

CHAPTER 5 Investigating the genetics of complex disease

5.1 The genetics of complex disease

The nucleus of each human cell contains the same tightly compact deoxyribonucleic acid (DNA) molecules that make up our genome. DNA is a polymer molecule made up of four sub-units- adenine, cytosine, guanine and thymine (A,C,G,T). The sequence of these DNA base pairs carries our genetic information. In 2001, the Human Genome Project published the first draft sequence of the human genome. This sequence was a consensus sequence showing what a typical human genome sequence looks like. It did not inform us of genetic differences between individuals. Following on from this, the International HapMap project is working to describe all single nucleotide positions where at least 5% of the population differs from the consensus genome and since 2008 the 1,000 Genomes project aims to discover >95 % of the variants with minor allele frequencies as low as 1% across the genome. By characterizing the genotypic variation in humans, genetic researchers seek to understand how genotypic variation relates to phenotypic variation.

A complex or multi-factorial disease is a disease that does not exhibit classic Mendelian dominant or recessive inheritance attributable to a single gene locus, but is determined by a number of genetic and environmental factors. These diseases are common and usually occur later in life. (eg. Hypertension; diabetes) There have been many technologies and strategies used to detect genetic influences of complex disease all of which have

inherent limitations.(Schork 1997) The genetic influence in complex disease is multi-factorial and can involve:

1 – Classical polygenic inheritance, in which a number of mutations at different loci must be present to result in disease. Polygenic traits may be classified as discrete traits which are measured by a specific outcome (such as death from myocardial infarction) or quantitative traits which are measured by a continuous variable (eg. Blood pressure).

2 – Genetic heterogeneity, in which defects in a number of genetic loci confer disease susceptibility independent of each other.

3 – Epistatis (gene interaction) in which the possession of a mutation will confer susceptibility to a degree dictated by the presence of other mutations.

4 – Environmental vulnerability and gene-environment interactions in which gene products are influenced by environmental stimuli and some may only have a deleterious effect in the presence of certain environmental factors.

5 – Incomplete penetrance and phenocopy – Some individuals who inherit a predisposing allele may not manifest the disease (incomplete penetrance) and others who do not inherit a predisposing allele may get the disease as a result of environmental or other influencing causes.

6 – Aging, in which with age, genes either mutate more rapidly or produce sub-optimal end products resulting in weakened or damaged physiologic mechanisms.

These features make the isolation of genes influencing complex disease difficult since the individual contribution may be obscured by the others or may exhibit dependency, where genetic effects may only occur in the presence of other factors. (Lander & Schork 1994; Schork 1997)

There are two basic strategies for identifying genes contributing to complex disease: Candidate gene analysis and whole-genome searches.

5.2 Candidate gene analysis

Candidate gene analysis involves testing the association between a particular genetic variant (allele) and a disease. If the particular genetic variant is more frequent in people with the disease than those without, it may either be a causative variant or may be related to a disease causing gene in a nearby locus. These analyses require prior information regarding the 'candidate' gene. This information usually derives from biological insights, similarity to other genes or deductive hypotheses. These studies are difficult in situations where little is known about candidate genes or if significant genetic heterogeneity occurs.(Schork 1997)

5.3 Linkage Analysis

Using evenly distributed genetic markers and knowledge of genetic linkage (where genes located close to each other on the same chromosome are inherited together more often than by chance), linkage analysis attempts to identify a genetic locus associated with a particular disease by identifying which alleles in the loci are segregating (being transmitted together) with the disease in families. If a set of marker alleles are segregating with the disease, they are said to be located near the disease gene. A likelihood of linkage ratio (LOD) is calculated to indicate statistical significance. Linkage analyses involve proposing a model to explain the inheritance pattern of phenotypes and genotypes observed in a pedigree. This method has worked well for monogenic Mendelian inheritance as there are few possible models

which may be easily tested. The applications to complex traits are more problematic as they may not fit a precise Mendelian inheritance model. Most linkage strategies are weak in detecting genes with small to moderate effect sizes and are rarely designed to assess multiple gene and environmental effects.(Lander & Schork 1994; Schork 1997; Mayeux 2005)

5.4 Common Disease/Common Variant theory and association studies

The principle of a genetic association study is to determine if there is a statistically significant difference in the frequency of one or more genetic markers between two groups. This is commonly tested between unrelated population-based cases and controls.(Orr & Chanock 2008) Association studies are based on an evolutionary theory, the common disease/common variant theory (CDCV), which hypothesizes that the genetic variation underlying susceptibility to common heritable diseases existed in the founding population of contemporary humans, such that the genetic variants predisposing to common disease arise on an 'ancestral genetic backbone' that is shared by a large proportion of those affected.(Reich & Lander 2001) Rare Mendelian inherited diseases which often result in strongly deleterious phenotypes would have been naturally selected out of the population. Common diseases often occur later in life and have little or no impact on reproductive fitness. Thus, common disease susceptibility alleles, rather than being selected out of the population, have persisted in moderate frequency because the human population has undergone a rapid and recent expansion. Alleles which may have been neutral or advantageous during human evolution may now confer susceptibility to disease because of a change in

the environment or living conditions that now results in disease manifestation.(Doris 2002; Altshuler *et al.* 2008)This theory hypothesizes that the genetic profile of common complex diseases is determined by genetic variants that are common in the population (allele frequency >0.05) and have individually a small effect on disease. Genome wide association studies are based upon this model and in design, they search for common variants of different allele frequencies between cases and controls.

Human Variation

Sequencing of the human genome has shown that the DNA sequence between 2 individuals from different populations is over 99% identical. However, the human genome includes over 3 billion base pairs, thus even with this high level of similarity between individuals, there are still more than 12 million sites of potential genetic variation between two individuals genome.(International HapMap Consortium 2005; Attia *et al.* 2009)Differences in genetic structure between individuals that occurs with a frequency of <1% are known as mutations; occurring more commonly than 1% are known as polymorphisms. These polymorphisms can take several forms. The presence (insertion) or absence (deletion) of an entire stretch of DNA, involving duplication of DNA is known as a copy number variation (CNV). Repeating patterns of DNA that vary in the number of repeats are known as Short Tandem Repeats (STR). Each repeating unit is usually between 200-300 base pairs in length and repeats several hundred times. Most commonly, a single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide (A, G, T, C) in the genome differs between individuals. Thus, SNPs describe locations in a chromosome where a single base varies between different people. These differences in individual bases are the most stable and common type of genetic variation

occurring in approximately 1 in 1000 base pairs. (2005; Doris 2002)They represent approximately 90% of common human genetic variation. Some SNPs are located in parts of the gene that code for a particular protein. In this case, they are known as non synonymous SNPs as they lead to a change in the amino acid sequence of the resulting protein, whereas synonymous SNPs do not result in an amino acid change. Other SNPs are not found in coding regions of DNA but may have an influencing effect on nearby genes. The most common nomenclature for SNPs refers to their reference SNP (rs) number eg. rs1837289. The presence of particular SNP allele in an individual is determined by testing ('genotyping') a DNA sample. The genotyping of random or selected SNPs in a group of unrelated cases and controls is known as a Genome Wide Association Study.

5.5 Genome Wide Association

Genome wide association studies (GWAS) are the method of choice for identifying common genetic variants predisposing to complex disease. They permit an unbiased and comprehensive analysis of the genome with the potential to identify novel susceptibility factors. These association studies are generally model free with no assumptions of the mode of inheritance and seek to determine whether or not a specific allele is associated with a disease.(Iles 2008a)

The fundamental principle of GWAS is that common diseases must be in part caused by common mutations. The most common mutation in the human genome is a SNP; a single base pair change between people occurring approximately every 1000 bases.(Altshuler *et al.* 2008) A GWAS depends on the identification of genetic variants in the form of SNPs and have been made possible by the International HapMap project and the rapid

development of massive parallel genotyping. The International HapMap Project is a large international collaboration that began in 2002 and seeks to determine the common patterns of DNA sequence variation in the human genome. It has done this by characterizing sequence variants, their frequencies and the correlations between them in DNA samples from populations with ancestry from parts of Africa, Asia and Europe. Similar to the human genome project in scope, it was a huge and successful collaboration that made all data freely available in the public domain.(International HapMap Consortium 2005) This catalog of genetic variation has provided the necessary 'blueprint' of human genetic variation making GWAS possible. GWAS seek to identify if the differences in common genetic variants between cases with a particular disease phenotype, and controls without are true disease associated variants. Since the early part of the 21st century, rapid advances have been made in genotyping technology and several commercial companies (eg. Illumina and Affymetrix) now provide high density SNP genotyping of between 200,000 SNPs up to 1.2 million SNPs. The methods of SNP selection differs between manufacturers, but Illumina and Affymetrix genotyping platforms (with greater than 300,000 SNP markers) both provide an estimated >75% coverage of common HapMap SNPs with an allelic correlation of 80% in Caucasians of European ancestry.(Barrett & Cardon 2006)

In GWAS, a large set of SNPs (>100,000) is genotyped across the human genome to determine the most common genetic variations that have a role in disease or to identify heritable quantitative traits that are risk factors for the disease.(Hirschhorn & Daly 2005) Commonly, GWAS use a case-control study design with a large number of participants, comparing the frequency of SNPs in a defined group of individuals with the disease and a similarly

matched group without the disease. If certain genetic variations are found to be significantly more frequent in people with the disease compared to people without, these variants may either be causative or more commonly may be associated with the causative mutation.(Satagopan & Elston 2003; Iles 2008a) By careful population selection and stringently phenotyping cases and controls, this approach has been highly successful identifying new genetic variants predisposing to medical disease.(Tenesa *et al.* 2008; Klein *et al.* 2005; Vitart *et al.* 2008)

5.6 DNA pooling

Although GWAS are a powerful and useful approach to detect new genetic variants contributing to disease, their cost can be prohibitively high. DNA pooling is a method designed to improve genotyping efficiency whilst trying to minimise loss of phenotypic and genotypic information. Using a DNA pooling strategy, pooled DNA from a group of cases and pooled DNA from a group of controls are formed. The allele frequency of each marker is then estimated by genotyping case and control pools instead of individual subjects, markedly reducing the number of genotyping assays performed.(Zhao & Wang 2009) DNA pooled GWAS are a viable alternative for screening disorders with common variations of large effect and have been demonstrated as a reliable and valid initial screening tool. DNA pooling has been successfully used to identify susceptibility loci in genome-wide association studies of neuroticism, schizophrenia and supra-nuclear palsy.(Shifman *et al.* 2008; Kirov *et al.* 2008; Melquist *et al.* 2007) However, apart from the challenges of analysis, DNA pooling does also have inherent limitations such as the introduction of experimental error in

formation of DNA pools (eg. accuracy of pool construction, integrity of pooled genomic data, unequal allele amplification) and within pool genotyping measurement variance which may necessitate several replicate measurements to reduce standard error.(Macgregor 2007; Barratt *et al.* 2002)

5.7 Next generation sequencing

While GWAS focus on identifying common SNPs which have a role in disease pathogenesis, advances in the understanding of the genome and in next-generation sequencing (NGS) technologies have permitted the study of rarer risk alleles that may influence complex disease.(Metzker 2010) NGS aims to sequence all base pairs in the genome providing a resolution that has not previously been possible. NGS technology is a rapidly evolving field that is constantly changing.(Cirulli & Goldstein 2010) New NGS technologies read DNA templates randomly along the entire genome and synthesise DNA in short reads of overlapping segments approximately 50-500 basepairs. To ensure the correct identification of genetic variants, short-read coverage must be sufficient to ensure the complete and accurate sequence assembly, and currently, at least 30 fold coverage is recommended in whole-genome scans for rare genetic variants.(Zhang *et al.* 2011) In the near future, NGS is predicted to become the best method of disease gene identification in large population based case-control studies, however the challenges in bioinformatics, data analysis and cost remain significant obstacles.(Faita *et al.* 2012) To overcome some of these challenges, geneticists have adopted different approaches such as targeted exome sequencing, sequencing of families or of phenotypic extremes.(Shi *et al.* 2011; Bras & Singleton 2011) In the last few years, reports of novel mutations in unknown disease and a move towards NGS as a diagnostic tool have been emerging, making this a

very exciting and promising tool in the future of clinical genetics.(Desai & Jere 2012; Shanks *et al.* 2012; Davies *et al.* 2012)

5.8 Genetic association studies

Genetic association studies may be designed to examine direct polymorphism association or indirect association.(Cordell & Clayton 2005)

5.8.1 Direct association

Direct association studies are designed so that polymorphisms studied are themselves putative causal variants. This is the most powerful type of association study and the easiest to interpret, however it requires a prior hypothesis about functional variants which may have a biological relation to the disease process. In practice, a set of SNPs are decided upon in regions of likely candidate genes and are used to test association in well characterised groups of cases and controls.(Cordell & Clayton 2005; Doris 2002; Campbell & Rudan 2007)

5.8.2 Indirect association

Because the odds of selecting the true causative SNP for direct association are impossibly low, indirect association studies are the predominant method of GWAS interpretation. In an indirect association approach there is no prior hypothesis, and polymorphisms may be in linkage disequilibrium with the true polymorphism conferring genetic susceptibility. These studies are weaker and more complex to interpret than direct association studies and they depend on the accurate knowledge of linkage disequilibrium patterns between associated markers within specific populations. (Cordell & Clayton 2005; Doris 2002; Campbell & Rudan 2007)

5.8.3 Linkage disequilibrium

When two alleles are statistically independent of each other they are said to be in linkage equilibrium. This is more likely to occur if the two alleles are far apart on the same chromosome. However when loci are sufficiently close together or are transmitted together, there is a statistical correlation between the alleles at each locus. This correlation is known as linkage disequilibrium (LD).(Conrad D.F. & Pritchard J.K. 2007; Orr & Chanock 2008) This allelic association may be interrupted in several ways such as gene conversion and by recombination events resulting in crossing over.(Myers *et al.* 2005) LD reflects human population history because its magnitude is determined by the level of recombination, migration, admixture, selection and random drift over successive generations.(Altshuler *et al.* 2008) If LD is well preserved, a single marker within a region of LD may be sufficient to give information on the adjacent polymorphisms. If LD is low or mixed, denser SNP markers are needed for more extensive coverage of the genome.(Doris 2002) When LD is plotted across the genome, it creates blocks of common genetic variation separated by so-called hotspots, where recombination rates are much higher than is usual. These blocks of common genetic variation are known as haplotypes. The characterisation of population specific haplotypes was carried out by the International HapMap Project, and the size and variation of haplotype blocks is critical in the analysis of GWAS. Haplotype blocks vary between population groups and have been shown to be smaller in Africans than in other populations.(Orr & Chanock 2008; Reich & Lander 2001; International HapMap Consortium 2005)

5.8.4 Quantifying LD in the Genome

A large number of statistical measures have been proposed as ways to quantify the level of LD between alleles. The three most common measures are: D, D' and r^2. D is the most basic measure of LD and represents the difference between the observed frequency of a haplotype from the expected frequency. A haplotype describes a sequence of alleles coming from the same chromosome. D takes a value of zero when there is no LD. D' is a standardised form of D (D/Dmax) and has a value between -1 and 1. A value of 1 represents complete LD. A maximum D' of 1 is reached when less than a total of four possible two locus haplotypes is observed in a population. r^2 represents the correlation co-efficient between two alleles at different loci. r^2 = 1 (perfect LD) if there are only two locus haplotypes present. As it is prohibitively expensive at present to perform whole genome sequencing, the knowledge of LD patterns in the genome allow the use of 'tagSNPs', where chosen SNPs within an LD block provide information about nearby SNPs without needing to genotype them. TagSNPs are usually selected so that they tag common nearby SNPs with an r^2>0.8. (Conrad D.F. & Pritchard J.K. 2007; Orr & Chanock 2008; Pritchard & Przeworski 2001)

5.9 Design of Genome Wide Association Studies

5.9.1 Case selection

Case ascertainment in GWAS is an important consideration in planning a successful study. Methods of case ascertainment can sometimes improve study power to find a disease causing allele by selecting cases from the extremes of clinical phenotypes or those cases with early onset disease or from large well characterized families. In addition, it is important that case definition is consistent particularly if multiple recruitment sites are involved

and that cases derive from the same or a similar population. (McCarthy *et al.* 2008b)

5.9.2 Control selection

Increasingly, common control databases are being used in GWAS. This method was successfully used in the Wellcome Trust Case Control Consortium (WTCCC) where controls were derived from a U.K. birth cohort and sampling of blood donors. Differences in survival and ascertainment between the two groups did not result in any spurious or significant associations indicating that perhaps their effect on genotype distributions were overestimated.(2007b) Nevertheless, disease prevalence and age of onset are important considerations in a control group. Certain common diseases may have a high prevalence in unselected controls which will reduce effective study power. Late-onset disease may cause misclassification bias, as susceptible individuals who have not yet reached the 'at risk' age period may be wrongly classified in a control group.

5.9.3 Two – stage design

Most current GWASs typically adopt a 2-stage design. When first established, conducting a well powered GWAS was prohibitively expensive for many scientific laboratories. Satagopan et al(Satagopan & Elston 2003) demonstrated the effective use of a 2 stage study as a design methodology that significantly reduced the quantity of genotyping needed and maintained study power. This method involves genotyping all cases and controls with a dense genome wide array in the first stage and selecting the most statistically significant associations from the analysis to genotype in a second (often larger) group of cases and controls. Significant polymorphism associations after this stage are then replicated in larger sample sizes or in

different case-control population groups as the best way to confirm a true association.(Igl *et al.* 2009)

5.10 Genome wide association studies– Error and bias

Although the design and analysis of GWAS are becoming more standardised, careful consideration should be given to the potential sources of error in GWAS.

The first step of GWAS is the whole genome amplification of the DNA and then hybridisation of DNA samples to the arrays.(Fan *et al.* 2006) Illumina hybridisation contains specific probes bound to beads (BeadChip). Their genotyping technology is based on the subsequent detection of fluorescently labeled molecules hybridized to these beads which are immobilized on a micro chip array. Genotypes are then called specific to different arrays by an automated algorithm. Defining a 'cut-off' genotype call rate is an important consideration. An excessively stringent cut-off call rate can lead to a loss of many SNPs (with a low call rate) such that true signals may be lost, and secondly, a potential rise in type 1 error (incorrectly rejecting the null hypothesis) , where differences in the pattern of missing genotype calls between cases and controls may result in a false association, a concept known a informative missingness.(McCarthy *et al.* 2008b) Too low a threshold reduces these problems at the expense of reduced accuracy and a higher chance of poorly called SNPs (due to technical error) being misrepresented in the association signals. Overall, a prudent approach appears to be more relaxed thresholds of genotype calling, followed by a careful assessment of other quality control measures and the inspection of signal genotype clusters. (McCarthy *et al.* 2008b; McCarthy & Hirschhorn 2008; Sebastiani *et al.* 2009)Other sources of laboratory error include

inefficient sample tracking, sample swaps and sample duplication. A standard way to identify some of these errors is to examine the agreement between phenotype gender classification and genotype by examining the heterozygosity of SNPs on the X chromosome and genotypically inferring gender. Samples with mis-matching gender assignments are usually removed from the analysis.

5.10.1 Population Stratification

An important potential source of confounding and significant cause of spurious associations in GWAS is due to population structure. This problem arises when cases (or controls) disproportionately represent a genetic subgroup, such that particular allele frequencies are more common within this group when compared to the general population. This may be due to a true association, but could equally be caused by different case and control ethnicities or relatedness or even hidden population substructure. (Freedman *et al.* 2004; Cordell & Clayton 2005) Several methods have been proposed to control for this potential source of confounding, however carefully selected cases and controls from a similar region of origin with the same ancestral ethnicity remains one of the best ways to reduce this source of error. (Hoggart *et al.* 2003) (Bacanu *et al.* 2000)A commonly used genomic method to identify and reduce population stratification is by examining genome wide identity by state (IBS). Individuals that are identical by state contain an independent copy of a particular allele. Individuals that share identity by descent (IBD) contain identical copies of the same ancestral allele.(Sebastiani *et al.* 2009) (see Figure 5.1) On the basis of the genomewide average proportion of alleles shared identical by state between any two individuals there are several GWAS analysis tools (eg. PLINK(Purcell *et al.* 2007)) that cluster individuals into subsets and

perform multidimensional scaling (MDS) which provides indices of population genetic variation (principal components). By plotting these principal components, outlying individuals representing population substructure can be identified. Similarly, by scoring the genetic similarity between every pair of subjects using estimates of the frequencies of alleles that are the same, a probability estimate for relatedness among study subjects can be calculated. (pi-hat) Individuals showing hidden relatedness are usually excluded in a case-control population design study.

5.10.2Hardy-Weinberg Equilibrium

An allele is one form of a DNA sequence in a gene at a particular site on a chromosome. As all humans have 2 pairs of chromosomes, an individual is said to have a heterozygous genotype at a locus if they have two different alleles and homozygous if they have two identical alleles. In 1908 Hardy and Weinberg described the mathematical relationship between genotypes and alleles representing a genetic trait. This relationship is known as the Hardy-Weinberg Equilibrium(HWE) and describes the relationship between the genotype frequencies from the allele frequencies. The law states that for two alleles *A* and *a* with frequency *p* and *q*. Then the distribution of these alleles is described by $p^2+2pq+q^2=1$ as long as there are no forces such as selection or migration that affect the frequencies and there is random mating in the population.(Hardy 1908) In GWAS it is important to check that observed allele frequencies in a population based study are in expected HWE proportions. This is observed by plotting a quantile-quantile(QQ) plot of the observed and expected allele frequencies. If there is deviation from the line (of null hypothesis) throughout, this may represent population stratification, relatedness or genotyping error. Deviation at the end of the range may

indicate large-effect susceptibility loci.(McCarthy *et al.* 2008b; Attia *et al.* 2009; Sebastiani *et al.* 2009)

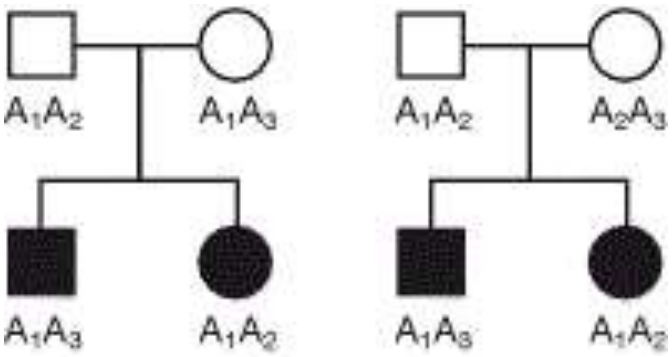


Figure 5.1- Identity by state (IBS) and identity by descent (IBD) - Both sib pairs share allele A_1. The first sib pair have two independent copies of A_1 (IBS but not IBD); the second sib pair share copies of the same paternal A_1 allele (IBD).

5.10.3Association testing

The most common analysis of SNP genotypes and case-control status is a per SNP χ^2 test of independence in a 2X3 contingency table. (cases and controls vs the two homozygote genotypes and the one heterozygote genotype) This statistical approach estimates a P-value at a particular threshold (usually α =5%), above which the null hypothesis of no association is rejected.(Balding 2006) Other investigators have suggested using the Cochrane-Armitage trend test with 1 degree of freedom, which improves power to detect an association in an additive model. (ie. Where two copies of a variant confers a higher risk than one copy)(Cardon & Bell 2001) In situations where genotype frequencies are small (<5), a Fisher exact test is appropriate. The limitation of all these tests is that they cannot include information about phenotype covariates. In this case, logistic regression is used to model the log odds for association to calculate a genetic effect odds ratio.(Balding 2006; Sebastiani *et al.* 2009) The regression equation is robust as it includes covariates (such as age and gender) and can also be

extended to incorporate gene-environment and gene-gene interactions. The other principal statistical approach involves Bayesian statistics. Bayesian statistics are based upon assuming a prior probability on the hypothesis of association or no association and then to predict the posterior probability of an unknown given the data. At present in GWAS this approach is not widely performed and is computationally intensive.(Lunn *et al.* 2006)

5.10.4Multiple testing

The adjustment for multiple testing in GWAS is an important component of analysis. The significance level for association is usually at an α of 5%. Thus all the performed one SNP association tests together should not have a probability of a false positive greater than α. To correct for this a Bonferroni correction is applied depending on the number of independent tests performed: To achieve an α= 5% if conducting 100,000 independent tests the actual threshold for genome wide significance of association is $P<5X10^{-7}$. Another method of estimating the type-1 error rate is to use permutation. This procedure is commonly used and involves randomising the phenotype labels but keeping the same genotype data. By running several analyses, a false-positive rate can be calculated.(Balding 2006)

5.10.5Imputation

Imputation describes the process of predicting or 'imputing' genotypes that are not directly assayed in individuals. In GWAS this refers to using a reference set of densely genotyped haplotypes (HapMap release reference) to impute into a study of individuals that have had a subset of these SNPs genotyped. In unrelated individuals, haplotypes over short distances will be related to each other by identity-by-descent (IBD).Haplotypes describe a sequence of genotype alleles on one chromosome copy, haplotype phasing is

a process that describes the two corresponding haplotypes for each individual given a set of genotypes. (Figure 5.2) Individuals that are homozygous at one or both loci have defined haplotypes, it is only when an individual is heterozygous at both loci that the phase is ambiguous. The haplotypes of each individual over short distances are phased and modeled as a mosaic of those in the haplotype reference panel (HapMap). After DNA strand alignment, the reference haplotype is the used to infer the missing genotypes and calculate a probability distribution. The main advantage of imputation, is that it can extend genomic coverage, allowing more tests for association and thus improving study power to detect association. However, the drawback is that imputed genotypes have not been directly assayed and provide only a likelihood score.(Marchini & Howie 2010; Kong *et al.* 2008)

	AA	**AT**	**TT**
GG	AG AG	AG TG	TG TG
GC	AG AC	AG TC or AC TG	TG TC
CC	AC AC	AC TC	TC TC

Figure 5.2 – Example – A given locus has alleles A and T with three possible genotypes AA, AT, and TT, the second locus has G and C, also giving three possible genotypes GG, GC, and CC. For a given individual, there are nine possible configurations for the genotypes at these two loci and eight corresponding haplotypes in the square that these resolve to.

CHAPTER 6 Clinical epidemiology of RRD in Scotland: Methodology

6.1 Study Population

Scotland is a well defined geographic region with a stable population and a highly developed infrastructure and healthcare system which provides an rare opportunity to study disease incidence with great accuracy.

This study was conducted in all six vitreo-retinal surgical sites in Scotland responsible for managing and administering surgical vitreo-retinal ophthalmic care for the population of Scotland. Data was collected between the 1st November 2007 and the 31st October 2009. The study participants are derived from the entire resident population of Scotland. The last formal Scottish census was conducted in April 2001 and was administered over one day. Data was gathered on all usual residents (excluding visitors) in Scotland. It was a highly accurate census with an overall response rate of 96% and an estimated coverage of 100%. Annual mid-year estimates (by age and gender) are calculated by the General Register Office of Scotland using a demographic cohort component method. Mid-year population estimates for 2008 were used in the analysis. The total population in 2008 was 5,168,500 (Males – 2,500,205; Females – 2,668,295) (http://www.gro-scotland.gov.uk/statistics/publications-and-data/population-estimates/mid-2008-population-estimates-scotland/index.html)

Fourteen health boards are established in Scotland to administer the Scottish health service. These health boards are divided geographically (See Figure 6.1) and all diagnosed RRD cases in Scotland are referred to one of six vitreo-retinal surgical sites for assessment and surgery. Figure 6.2 and 6.3 highlight the age, gender and health board distribution of the Scottish population according to the 2008 mid-year estimates.

6.2 Participating centres

The six vireo-retinal surgical sites (Ayr Hospital, Ayr, Gartnavel General Hospital, Glasgow, Princess Alexandra Eye Pavilion, Edinburgh, Ninewells Hospital, Dundee, Aberdeen Royal Infirmary, Aberdeen and Raigmore Hospital, Inverness) are geographically distributed across 14 different health boards and they are responsible for and manage the operative workload of all cases requiring retinal surgery throughout Scotland. Each identified case in Scotland is referred to one of these centres for assessment and further management. There are 16 vitreo-retinal consultant specialists in Scotland who helped to co-ordinate this study.

6.3 Ethical aspects

Multi-centre research ethics committee (Scotland) (06/MRE00/19) and NHS Trust management committee approval (MRAD07/OP01/DI) were obtained from all participating sites. Individual informed consent was received on the basis that each participant understood and had time to consider the merits of study participation; participation was voluntary and did not affect subsequent care of the patient; the DNA sample and clinical data about the individual would be stored by the research team at Edinburgh University for use in future research and may be shared with other medical research groups (with appropriate ethical approvals first being obtained where necessary). All data was recorded and stored in compliance with ethical and data protection guidelines.

6.4 Case Definition

All incident cases of primary rhegmatogenous retinal detachment (RRD) presenting to all participating centres were invited for study inclusion. The diagnosis of RRD was based on a case definition of "a full thickness break in the neurosensory retina with a surrounding area of sub-retinal fluid extending greater than 2 disc diameters" (Zou *et al.* 2002; Schepens 1951; Li 2003; Polkinghorne & Craig 2004b). No distinction was made in this study between clinical and subclinical (asymptomatic) retinal detachment, all diagnosed cases were invited.(Schepens 1951) As the original definition shows observer dependant bias, I extended RRD classification in surgical terms as any break in the neurosensory retina with surrounding sub-retinal fluid requiring intervention more than cryotherapy and/or laser treatment to reattach or stabilise the retina. Thus in practice any patient who had a RRD of significant size which was treated with barrier laser alone as well as those who underwent an operative procedure including vitrectomy and internal tamponade, pneumoretinopexy or external buckle were included. Patients were also included if they presented with a RRD but for medical, social or occupational reasons the decision to perform surgery was not taken. Cases of a retinal tear and associated vitreous hemorrhage where a vitrectomy and internal tamponade procedure was performed were excluded. Blunt traumatic cases of RRD were included, penetrating trauma was excluded. All other types of retinal detachment (exudative, tractional and combined) were excluded. Re-detachments regardless of duration of attachment post-operatively were excluded.

Three conditions must be satisfied to cause RRD: vitreous liquefaction, tractional forces producing a full thickness retinal break and an open retinal break allowing fluid to gain access to the sub-retinal space.(Ghazi & Green

2002) By convention, the subtype of RRD is described according to the type of retinal break causing the detachment and may be classified as follows:

Horse-shoe shaped tear (HST) – This refers to tear in the neurosensory retina due to a persistent site of vitreo-retinal traction which occurs during a posterior vitreous detachment.

Giant Retinal Tear (GRT) - This is defined as circumferential retinal break of 90 degrees or more. The tear is usually along the posterior margin of the vitreous base, may have a rolled edge and rarely may occur at the anterior margin through the ciliary epithelium.(Michels RG *et al.* 1990)

Dialysis – Defined as circumferential tears along the ora serrata with persistent vitreous attachment to the posterior retina (ie. Incomplete posterior vitreous detachment).(Hagler 1980)

Round Hole – This refers to an avulsed or operculated area of peripheral retina induced by a complete or localised vitreous separation. It may also refer to atrophic retinal holes associated with areas of pigmentation or peripheral retinal degeneration which may be present with or without an associated PVD.

Retinoschisis – This describes a degenerative process causing a splitting of the retinal layers. If retinal breaks occurs in both the outer and inner leaves of a retinoschisis causing a progressive extension of sub-retinal fluid requiring intervention this is classified as a retinoschisis detachment (Straatsma & Foss 1973).

6.5 Case identification and recruitment

Cases eligible for recruitment were confirmed through clinical examination by a retinal consultant. After informed consent, a detailed history and clinical examination was performed on all cases personally or by a senior

ophthalmologist (consultant or vitreo-retinal fellow). Where there was ambiguity over clinical characterisation, the most senior opinion was used. Blood sampling was performed on all consenting participants. The principal method of data collection involved daily to weekly personal visits to each participating centre. I attended pre and post operative ward rounds on a weekly or twice weekly basis in the busiest centres, namely Edinburgh, Glasgow and Dundee. The other centres (Ayr, Aberdeen, Inverness) were visited every 2-3 weeks. In these peripheral centres, I liaised with an appointed local investigator or vitreo-retinal consultant on a weekly basis to ensure complete case capture. I also liaised on a weekly basis with the relevant secretarial department of each consultant vitreo-retinal surgeon in each centre to ensure that any discharge or post-operative letters dictated with 'retinal detachment' of any type as the principal diagnosis were kept for my examination. The majority of cases were identified and recruited on the day of surgery or on the first post-operative day. Further cases were identified through informal referrals, accident and emergency/ out-of-hours attendance and waiting list office registration.

6.5.1 Data Quality

A number of procedures were established to ensure optimal data quality. Firstly, at the outset, I visited each centre and established didactic tutorials with the retinal department staff and nominated local investigators. All nominated investigators were trained by demonstration and explanatory protocols on accurate completion of the questionnaires. I visited each site regularly to guarantee data completeness. If possible, all incident cases were identified and approached for inclusion while in hospital. Where cases were recruited pre-operatively, the clinical notes were examined post-

operatively and the extent of pathology discovered intra-operatively was recorded. Attempts were made to complete all data fields by directly contacting the optician or patient by telephone. In particular, to obtain the refractive error measurements of individuals who underwent cataract surgery prior to RRD presentation, I sent a self-addressed questionnaire to each individual requesting their earliest glasses prescription. If this was earlier than the date of cataract surgery in the detached eye, the refractive error was noted. If not, a 'missing' entry was recorded.

6.5.2 Data Validation

Several validation techniques were established to ensure complete case capture. Firstly, I examined surgical logbooks in each centre (where each operation is recorded in detail) on each visit to determine all cases that underwent vitreo-retinal surgery of any type. This list was then cross referenced with locally collected data during this time period to identify any potentially missed cases. Subsequently, I reviewed all out-patient letters, discharge letters and clinical case notes to determine how many cases met the inclusion criteria but were not recruited during the short time period between my visits.(e.g. 3 days or one week) I followed up any ambiguous or incomplete information by contacting the patient or surgeon directly. Cases that were missed during their in hospital stay but that met the inclusion criteria were invited for inclusion at the first post-operative follow up appointment. Each nominated local investigator was also asked to record information on any willing study participant who did not undergo surgery for medical or other reasons. The number of identified cases that were lost to follow up or that refused consent was recorded.

The data collection method established ensured, as close as possible, complete case capture of all symptomatic RRD cases presenting for treatment and all RRD cases that underwent surgical repair in Scotland. This methodology was not robust enough to accurately establish the incidence of asymptomatic RRD or cases which may not be referred for treatment. Similarly, patients presenting privately may have been missed. However with the availability and access to healthcare in Scotland and the well established pattern of referral in the NHS and highly developed transport infrastructure, I expect that in Scotland, this number is very small.

6.6 Data fields

Data fields gathered by the author comprised (see Appendix B for proforma):

1. *Demographic details*

Date of Birth
Date of attendance
Gender
Postcode
Ethnicity
Route of referral

2. Ocular/Medical parameters

Presenting complaint - nature and duration of symptoms
History of ocular trauma and type of trauma
Past medical history
Refractive status (prior to any ocular surgery surgery) – derived from optician letter, contact with the optician or through focimetry of participants' glasses.
Biometry – Average axial length measurement

Previous ophthalmic history- type of ocular condition, date of any previous intra-ocular surgery or prophylactic laser treatment, presence of complicated cataract surgery with vitreous loss, presence of YAG capsulotomy, features of Stickler syndrome.
Known parental consanguinity
Family history of eye disease
Family history of retinal detachment; A positive family history was considered to be any first degree relative affected by a retinal detachment which required treatment. (i.e. subclinical cases which did not warrant treatment were not considered
Type and date of RRD repair surgical procedure was noted

3. Biomicroscopic and clinical examination

Snellen chart corrected distance visual acuity
Applanation tonometer intra-ocular pressure measurement
Fundal examination – macula status; type, number and location of retinal breaks; presence and grade of proliferative vitreoretinopathy; clock hours of detachment, location and extent of detachment
Vitreous examination – partially detached; detached; optically empty; haemorrhage; membranous deposits Peripheral retina examination – Presence, type and location of any peripheral retinal degeneration.
Fellow eye examination – documentation of any observed vitreo-retinal pathology.

6.7 Examination protocol

Visual acuity

Visual acuity for distance was performed at presentation by a trained nurse practitioner using a Snellen chart at 6 meters. Best corrected visual acuity was measured separately for each eye and is recorded as the smallest line which the patient can accurately read with corrected refractive error. If the largest letter was not visualised (6/60), the examiner tests finger counting at one meter. The examiner then tests 'hand motion' detection at one meter

and finally light perception is tested by with a pen-torch light and a visual acuity of perception of light, or no perception of light is then recorded.

Slit lamp biomicroscopy

A slit lamp biomicroscopy examination was performed on all participants by a vitreoretinal specialist. After appropriate positioning of the patient, the anterior segment of each eye was examined and the structures of the anterior segment were inspected for any lesions or abnormalities which were then documented.

Applanation tonometry

Intra-ocular pressure was measured by a standard method using a Goldmann Applanation Tonometer.

Dilated fundal examination

All participants had both eyes dilated (tropicamide 1% and phenylephrine 2.5%) and a systematic examination of both fundi was performed by a consultant ophthalmologist or senior clinician. Examination was performed initially at the slit-lamp using a condensing lens and subsequently in the supine position using an indirect ophthalmoscope with scleral indentation. Each quadrant was examined systematically and all noted pathology was recorded on a standardised fundal drawing.

6.8 Ocular features defined

A myopic refractive error was defined as a spherical equivalent refractive error (SER) of ≥-1 dioptre (D), a hypermetropic refractive error as a spherical equivalent refractive error of ≥+1D and emmetropia as a spherical equivalent refractive error of less than -1 and less than +1D. Low myopia was defined as a SER of >-1D to <-6D. High myopia was defined as a spherical equivalent refractive error of >-6D. Low hypermetropia was

defined as a spherical equivalent refractive error of >+1D to <+6D and high hypermetropia was defined as a SER of >+6D.

Trauma was defined as any direct or indirect trauma to the eye resulting in loss or disturbance of vision warranting ophthalmological review.

Posterior vitreous detachment is due to progressive liquefaction of the aging vitreous which induces its separation from the inner-limiting membrane of the retina. This process may be partial or complete. The presence of a Weiss ring designated a complete PVD, its absence designated an incomplete PVD.(Sebag 1987)

Peripheral retinal degeneration delineates peripheral retinal lesions which cause anomalies in vitreo-retinal adhesion which may predispose to RRD. These lesions include pavingstone degeneration, retinal cysts, retinal tufts and white without pressure.

Lattice degeneration is characterised clinically as circumferentially distributed, sharply demarcated, elliptical areas of peripheral retinal thinning with variable pigmentation. There are often localised areas of overlying vitreous liquefaction, a criss-crossing network of hyalinised vessels and exaggerated vitreoretinal attachments along the edge of the lesion (Byer N.E 1979).

Proliferative vitreoretinopathy (PVR) – This is characterised as an anomalous scarring process due to growth and contraction of cellular membranes in the vitreous and on the retinal surface. An updated classification of the 1983 Retina Society Terminology Committee classification was used as a clinical guide to grade PVR.(Pastor 1998; Machemer *et al.* 1991)

6.9 Scottish Index of Multiple Deprivation

The Scottish Index of Multiple Deprivation (SIMD) is the Scottish Government's official tool for identifying small area concentrations of multiple deprivation across Scotland, which enables effective targeting of policies and funding. (http://www.scotland.gov.uk/Publications/2009/10/28104046/0) The SIMD approaches deprivation as a range of problems that arise due to lack of resources or opportunities and provides a multi-dimensional indicator of deprivation. It uses a total of 6,505 datazones, which are population based geographic areas with approximately 750 people living in each one. The datazone for each patient could be identified by their postcode. The SIMD ranks these areas from 1, the most deprived, to 6,505, the least deprived, providing a ranking for every datazone in Scotland. The datazones are ranked according to an overall deprivation score, which is a weighted sum of seven domain scores (current income, employment, health, education, geographic access, crime and housing) derived from 37 different indicators. The SIMD index provides a relative ranking and not an absolute measure of deprivation. (ie. The datazone ranked 50 is not twice as deprived as the one ranked 100). The domain weightings used in SIMD 2009, expressed as a percentage of the final score are: current income (28%), employment (28%), health (14%), education (14%), geographic access (9%), crime (5%) and housing (2%). Using the SIMD 2009 database I matched the postcode of each case to the ranked datazone to analyse the socio-economic profile of the cases.

6.10 Statistical methods

Annual RRD incidence rates and 95% confidence intervals were calculated for gender and age-group based on the Poisson distribution using the 2008 population mid-year estimates. Age-standardised incidences were calculated by a direct method using the European Standard Population reference. Differences in incidence between comparison groups were calculated based on Poisson distribution and exact p-values are reported. Proportionality differences, including trends, were calculated using chi-squared statistics. Meta-estimates were calculated based on a random effects model and displayed as a forest plot. All reported p-values are based on two-sided tests.

Figure 6.1 – Health board distribution in Scotland. (Courtesy of the National Registry Office, Scotland)

Age	Persons	Males	Females	Age	Persons	Males	Females	Age	Persons	Males	Females
All ages	**5,168,500**	**2,500,205**	**2,668,295**								
0	59,531	30,320	29,211	30	58,887	29,280	29,607	60	69,089	33,773	35,296
1	57,495	29,509	27,986	31	56,987	28,418	28,549	61	74,880	36,483	38,397
2	55,851	28,586	27,265	32	60,340	29,701	30,639	62	57,476	28,132	29,344
3	55,407	28,582	26,825	33	60,954	29,593	31,361	63	54,976	26,549	28,427
4	54,688	28,155	26,533	34	62,124	30,219	31,905	64	55,981	27,057	28,924
0 - 4	**282,972**	**145,152**	**137,820**	**30 - 34**	**299,272**	**147,211**	**152,061**	**60 - 64**	**312,382**	**151,994**	**160,388**
5	53,135	27,104	26,031	35	66,269	31,985	34,284	65	54,444	25,805	28,639
6	52,267	26,538	25,729	36	70,556	33,677	36,879	66	50,279	23,694	26,585
7	53,332	26,987	26,345	37	74,682	35,887	38,795	67	47,110	21,947	25,163
8	54,036	27,957	26,079	38	74,310	35,639	38,671	68	48,498	22,583	25,915
9	56,710	29,105	27,605	39	77,210	37,121	40,089	69	47,465	22,098	25,367
5 - 9	**269,480**	**137,691**	**131,789**	**35 - 39**	**363,027**	**174,309**	**188,718**	**65 - 69**	**247,796**	**116,127**	**131,669**
10	57,746	29,462	28,284	40	78,861	37,632	41,229	70	46,355	21,475	24,880
11	59,459	30,605	28,854	41	80,430	38,942	41,488	71	44,563	20,277	24,286
12	58,996	30,178	28,788	42	79,682	38,109	41,573	72	43,556	19,622	23,934
13	59,875	30,672	29,203	43	81,970	39,422	42,548	73	41,782	18,599	23,183
14	61,708	31,483	30,225	44	81,945	39,156	42,789	74	39,312	17,269	22,043
10 - 14	**297,754**	**152,400**	**145,354**	**40 - 44**	**402,888**	**193,261**	**209,627**	**70 - 74**	**215,568**	**97,242**	**118,326**
15	63,328	32,329	30,999	45	81,183	38,759	42,424	75	37,556	16,273	21,283
16	65,833	33,650	32,183	46	79,931	38,882	41,049	76	37,100	15,801	21,299
17	66,326	34,113	32,213	47	78,558	37,958	40,600	77	35,152	14,737	20,415
18	65,022	33,481	31,541	48	76,644	37,199	39,445	78	32,875	13,408	19,467
19	66,926	34,266	32,660	49	75,769	36,636	39,133	79	30,664	12,363	18,301
15 - 19	**327,435**	**167,839**	**159,596**	**45 - 49**	**392,085**	**189,434**	**202,651**	**75 - 79**	**173,347**	**72,582**	**100,765**
20	70,520	36,150	34,370	50	73,967	35,755	38,212	80	27,309	10,836	16,473
21	71,148	35,947	35,201	51	72,105	35,009	37,096	81	25,670	9,955	15,715
22	72,094	36,568	35,526	52	70,105	34,315	35,790	82	24,592	9,240	15,352
23	72,351	36,736	35,615	53	67,599	33,076	34,523	83	21,772	7,997	13,775
24	67,940	34,488	33,452	54	66,801	32,736	34,065	84	20,006	7,154	12,852
20 - 24	**354,053**	**179,889**	**174,164**	**50 - 54**	**350,577**	**170,891**	**179,686**	**80 - 84**	**119,349**	**45,182**	**74,167**
25	67,078	33,991	33,087	55	65,479	32,121	33,358	85	17,100	5,736	11,364
26	67,223	33,927	33,296	56	63,239	30,980	32,259	86	15,954	5,205	10,749
27	68,905	35,130	33,775	57	64,433	31,487	32,946	87	14,599	4,649	9,950
28	67,870	34,452	33,418	58	64,993	31,885	33,108	88	13,530	4,193	9,337
29	63,755	32,447	31,308	59	67,057	32,869	34,188	89	8,109	2,376	5,733
25 - 29	**334,831**	**169,947**	**164,884**	**55 - 59**	**325,201**	**159,342**	**165,859**	**85 - 89**	**69,292**	**22,159**	**47,133**
								90 & over	**31,191**	**7,553**	**23,638**

Age group summaries

	Persons	Males	Females
All aged under 16	913,534	467,572	445,962
All aged under 18	1,045,693	535,335	510,358
All aged 16 & over	4,254,966	2,032,633	2,222,333
All aged 18 & over	4,122,807	1,964,870	2,157,937
All aged 16-29	952,991	485,346	467,645
All aged 30-44	1,065,187	514,781	550,406
All aged 45-64 (M), 45-59 (F)	1,219,857	671,661	548,196
All aged 65 & over (M), 60 & over (F)	1,016,931	360,645	656,286
All aged 75 & over	393,179	147,476	245,703

Figure 6.2 – The age and gender distribution of all Scottish residents in Scotland based on the 2008 mid-year population estimate. (Courtesy of the National Regsitry Office, Scotland)

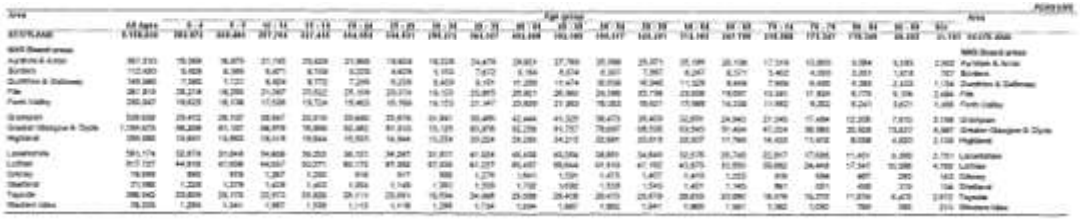


Figure 6.3 - The age and population structure of all Scottish residents in each health board region in the 2008 mid-year population estimate. (Courtesy of the National Regsitry Office, Scotland)
Note- Argyll and Clyde is now considered part of the Highland Health Board region.

CHAPTER 7 – Genome-wide association study of primary RRD: Methodology

7.1 Overall Design

The genome wide association study (GWAS) was designed in 2-stages to increase genotyping efficiency.(Satagopan & Elston 2003) In the first (discovery) stage, 912 prospectively recruited Scottish RRD cases were genotyped using the Illumina CNV370v3 Quad genotyping array and we compared the genotypic counts with 1,986 ethnicity matched Scottish controls that were previously genotyped using the Illumina Hap 300 and Hap 240S in an unrelated study.(Tenesa *et al.* 2008)

In stage 2 we genotyped the most significant 4,541 SNPs in 217 cases recruited in London, 470 cases recruited in Cambridge, 121 cases recruited in Scotland, and 264 cases recruited in Nijmegen, Holland. The phenotype and genotypic data of the 1958 Birth Cohort were used as a control group for the English cases (N=2,594). Nijmegen collaborators provided 326 age and gender matched local controls for analysis.

7.2 GWAS Power and sample size

In assessing study power, there should be sufficient power to detect genetic effects likely to be of biological and public health importance. Thus, it is important to be able to detect a genotypic risk ratio of ~1.5 with minor allele frequency (MAF) of >10%. Genotypic risk ratios for GWAS in common complex diseases have ranged fairly widely but most are in the range 1.1 – 1.7.(Iles 2008a) Using a case-control power calculator for a two stage

association study http://www.sph.umich.edu/csg/abecasis/CaTS/ and assuming perfect LD between marker and risk alleles I explored power calculations for a number of genotypic relative risk variants with an estimated disease prevalence of 0.003.(Iles 2008b) Figure 7.1 illustrates power for varying genotypic risk ratio and MAFs to detect a disease locus with multiplicative effect by a two-stage strategy with a significance level of $p<1x10^{-7}$. I estimate that a total of 2,000 RRD cases and 2,000 matched population-based controls in a two stage approach would give acceptable power to detect a genotypic risk ratio of approximately 1.5 at a MAF of 10-15% or greater.

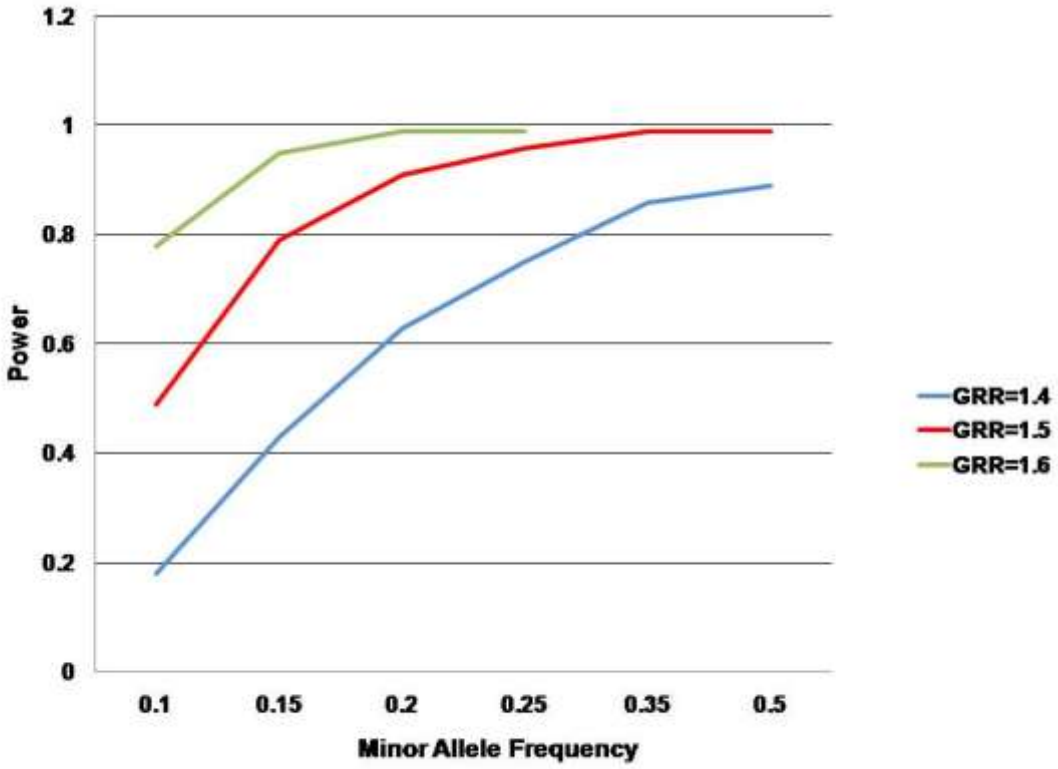


Figure 7.1 – Power estimates by genotypic relative risk (GRR) and allele frequency for a 2 stage genome wide association study with a total sample size of 2000 cases and controls.

7.3 Peripheral blood storage and DNA extraction

The peripheral blood sample from each consenting participant was stored in a freezer provided at each of the six recruitment sites in Scotland. I delivered samples on dry ice every 3 weeks to the Wellcome Trust Clinical Research Facility (WTCRF), Edinburgh. At the WTCRF, blood tubes were stored at -40°C until ready for extraction. 100µl of blood from each sample were spotted onto an FTA card (Whatman) and cards archived at room temperature in a safe. DNA extraction used the Nucleon Kit (Tepnel Life Science) with the BACC3 protocol. The precipitated DNA was hooked out and placed directly into a labeled 2.0ml microtube (Scientific Specialities Inc) containing 1.0 ml TE buffer pH 7.5 (10mM Tris-Cl pH 7.5, 1mM EDTA pH 8.0). Microtubes were rotated for 2 weeks until DNA was fully re-suspended. 8 out of 92 samples were electrophoresed on a 1% agarose gel to test for integrity of the DNA and were also tested via the Nanodrop method for protein contamination. DNA concentration (ng/µl) was measured using picogreen. DNA master stocks were split into 500µl in a deep well plate and 500µl in a microtube. DNA in the deep-well plate was then normalised to 50ng/µl and used to make working stock plates.

7.4 Stage 1

Figure 7.3 summarises the case- control comparison for stage 1 genotyping. Overall, we compared genotypes of 912 cases of primary rhegmatogenous retinal detachment with 1,986 Scottish individuals derived from the S.O.C.C.S study.(Tenesa *et al.* 2008)

7.4.1 Case selection

DNA was extracted on 1,139 cases of primary RRD recruited throughout Scotland between 31st October 2007 and 1st November 2009. For genotyping of stage 1 cases, all cases of known relatedness and of non-white British ethnicity were excluded. In addition, I developed further exclusion criteria in addition to ones previously outlined(Mitry *et al.* 2009b) based upon phenotypic variables in order to enrich our database for cases more likely to have a genetic or heritable component. Cases meeting the following criteria were excluded from the genetic analysis:

-Aphakia
-Complicated cataract surgery
-Direct ocular trauma
-Ocular/ systemic features of hereditary vitreoretinopathy or syndromic RRD
-Previous posterior segment intra-ocular surgery
-Previous uncomplicated cataract surgery presenting with RRD within 2 years from the time of initial cataract removal

In total, 912 cases of primary non-traumatic rhegmatogenous retinal detachment were selected for genotyping. These samples were genotyped between November 2009 and January 2010 at the Wellcome Trust Clincial Research Facility in Edinburgh using the Illumina 370 Quadv3 Beadstation array.

7.4.2 Control selection

Unrelated controls for the stage 1 genotyping analysis of this project comprised the cases and controls from an unrelated study, the phase 1 Scottish Colorectal Cancer Study (SOCCS).(Tenesa *et al.* 2008) The recruitment for the SOCCS study commenced in February 1999 and ended in December 2006.

Controls for the SOCCS study were randomly identified through the Community Health Index (CHI), which is a NHS population-based register. CHI is a national register of all individuals who are registered with a general practitioner (GP) in Scotland. The controls were drawn following a matching protocol applied to the CHI and they were recruited through clinics set up in over 40 locations across Scotland. Controls were selected at random and invitations passed on to these individuals via their GPs. Figure 7.2 below summarises the control recruitment algorithm protocol for the phase 1 SOCCS study.

Cases for the phase 1 SOCCS study were recruited from all tertiary treatment centres in Scotland between 1999 and 2006. All cases had a confirmed histological diagnosis of adenocarcinoma of the large bowel epithelium and were diagnosed with colorectal cancer at age $\leq$55yrs. Detailed demographic, clinical as well as environmental exposure information was collected on recruited cases and controls. The clinical data (self reported past medical and surgical history) of all SOCCS cases and controls was reviewed and individuals reporting either a history of retinal detachment or intra-ocular surgery for retinal detachment repair or of undetermined type were excluded from further analysis. Based on these criteria, one SOCCS control sample was excluded and seven SOCCS case samples were excluded.

In total genotypic data was used for 1,002 SOCCS controls and 983 SOCCS cases. The quality control thresholds for the exclusion of SOCCS individuals have been described in a previous publication.(Tenesa *et al.* 2008) In total, seven people were excluded due to non-Caucasian ethnicity, fourteen individuals with gender discrepancies, 5 individuals with hidden relatedness and fifteen individuals with genotype failure. This cohort was

genotyped using the Illumina HumanHap 300 and the Illumina HumanHap 240S in 2007.

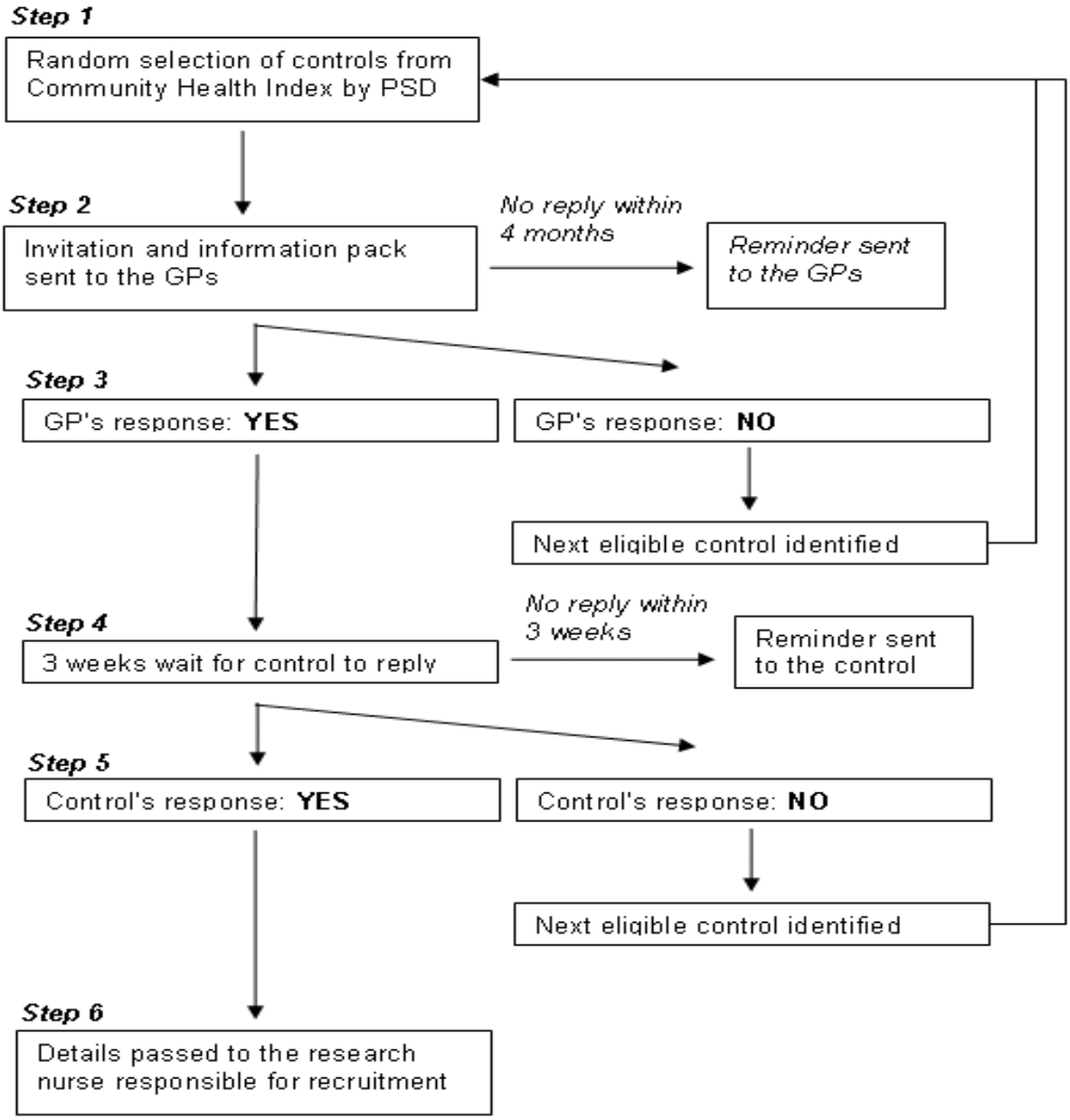


Figure 7.2 – Recruitment algorithm for SOCCS control participants

7.4.3 Suitability of cases and controls

The justification of control selection for stage one was based on several considerations. Firstly, the SOCCS participants were genotyped with the Illumina 300 and 240S genotyping platforms, which together provide over

95% coverage of SNP markers on the Illumina 370Quad array which we have used to genotype our cases. Approximately 50% of the SOCCS database however comprised selected individuals with a diagnosis of colorectal cancer. Although these individuals have been recruited according to a pathological diagnosis and specific age criteria, and may represent a possible source of population stratification, they have been recruited 'Scotland-wide' from the same Scottish referral centres as our cases. In addition there is no known clinical or etiological association between colorectal cancer and rhegmatogenous retinal detachment. Thus I concluded that it was reasonable to include these cases into the control group in our analysis to improve the study power for association. Colorectal cancer status was noted and included as a variable in the analysis model to determine if this inclusion did have an impact on any potential SNP association. Figure 7.4 highlights the age difference between the RRD cases and our SOCCS control group demonstrating the younger SOCCS group. Similarly there was a gender disparity between our cases and controls (RRD(N) cases: Male(513):Female(355) = 1.44:1 and SOCCS cases and controls(N): Male(1,003):Female(965)= 1.04:1 ; X^2 difference – 15.92;p<0.0001) However, based on the postcode region of residence between the RRD cases and SOCCS control group, there was no difference noted in the geographical region of residence between the two groups. (Figure 7.5)

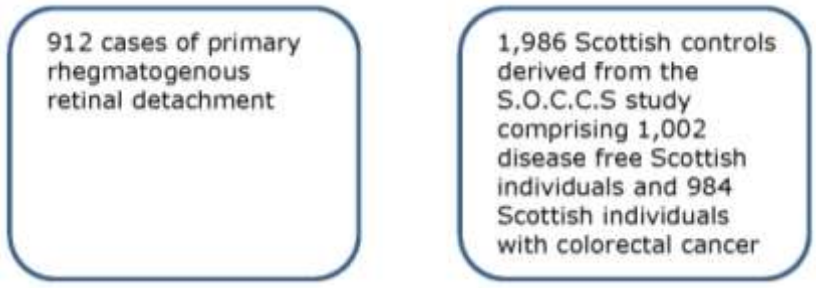


Figure 7.3 – The total genotypic case-control comparison for stage 1

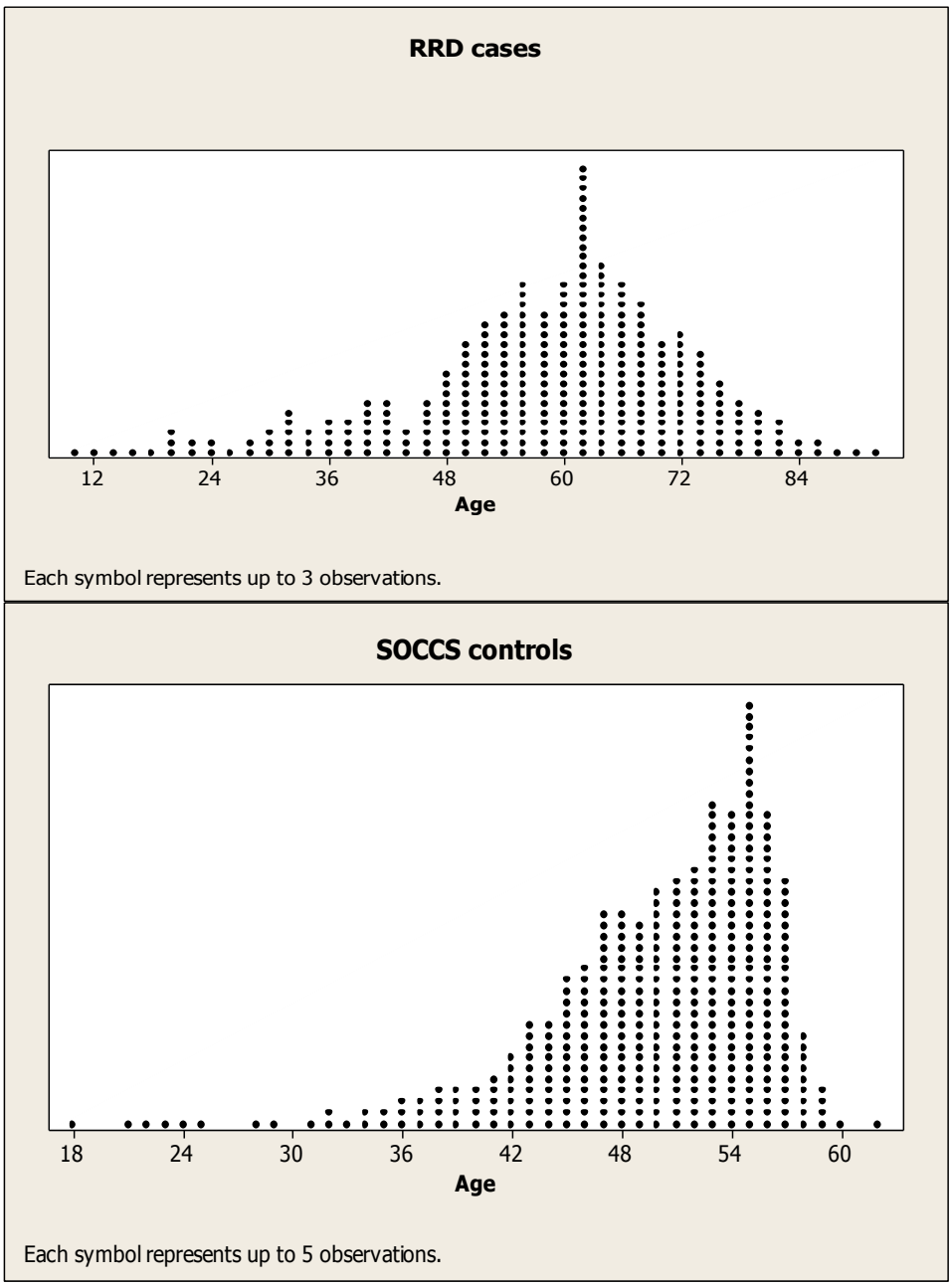


Figure 7.4 – Age distribution of RRD cases and all SOCCS a controls (RRD mean age(SD)=58.9(13.8); SOCCS controls mean age(SD)= 50.16(6.1); T=17.9;p<0.001)

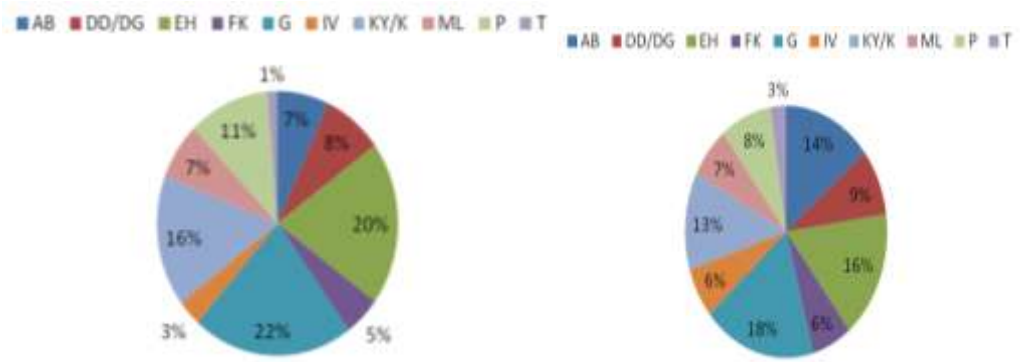


Figure 7.5 – The geographic distribution by the first two postcode letters of all RRD cases (left) and SOCCS controls (right) used in stage 1. There was no significant difference between proportions.

7.5 Illumina 370 Quad Genotyping Array

The RRD cases were genotyped using the Illumina CNV370v3 Quad genotyping array. There were several reasons behind this selection. Illumina was the commercial company chosen primarily to match the SNP markers used in the previously genotyped controls and because of previous experience with the high levels of genoptyping quality achieved. The CNV370v3 Quad array represents a compromise between financial limitations and genomic coverage, containing approximately 95% of SNP markers on the Illumina 300S and 240 HumanHap genotyping platform (which was used to genotype the SOCCS controls) allowing appropriate case-control comparison with a high degree of genome coverage.

7.6 Stage 2

To develop case collection in additional populations suitable for replication, I established a working collaboration with several other centres in the U.K. and Europe based upon the case eligibility criteria and the ethical principles of the Scottish study.

7.6.1 Case selection – London

I extended ethical and research and development approval to enable collection of cases of primary RRD from Moorfields Eye Hospital (MEH) in London. (MREC:06/MRE00/19 – Amendment date 5th May 2009) MEH has the highest throughput of cases in the U.K. and represents an important source of potential cases, which can be readily collected as all surgery for RRD cases is performed on-site. I commenced collection in January 2010 using the same inclusion criteria and proforma as in the Scottish RD Study making cases derived from both studies highly comparable. Blood samples were similarly stored in a locally provided freezer and were transported on dry ice each month to the Wellcome Trust Clinical Research Facility (WTCRF) in Edinburgh where DNA extraction was performed and DNA stored using the same protocol as for the Scottish cases.

7.6.2 Case selection – Cambridge

The retinal research unit in Addenbrookes Hospital in Cambridge (http://www.vitreoretinalservice.org/research.htmlitreo-retinal) is led by Mr Martin Snead and Mr Allan Richards, both of whom have a well established interested in the familial genetics of retinal detachment. I approached Mr. Snead about the project and together, we applied for ethical approval (Cambridgeshire REC 02/172- Amendment date 20th April 2009), and extended case collection using the same ethnicity and diagnostic criteria, to recruit unrelated population based cases from an established large clinical database. These cases were recruited from May 2009 onwards. DNA was stored and extracted in the molecular genetic laboratory at Addenbrookes Hospital and samples were transported to the WTCRF in Edinburgh where they were quantified using PicoGreen and normalized on plates for genotyping.

7.6.3 Case selection - Nijmegen, Holland

Professors Carel Hoyng and Anneke den Hollander from the Department of Human Genetics at the Radboud University Medical Centre in Nijmegen, Holland (http://www.ncmls.nl/NCMLS/MenuStructures/PI/theme3/AnnekeDenHollander.asp) have published extensively on the genetics of inherited retinal dystrophy. I approached this group outlining the principal aims of our project and we applied for ethical approval to extend case collection and recruit cases in Nijmegen. Case collection had been initiated in 2003 with the analysis of several pedigrees with inherited RRD.(Go *et al.* 2003) We extended case collection to recruit unrelated cases. All cases derived from Holland matched the clinical eligibility criteria outlined for the Scottish study and were of Dutch ethnicity. DNA samples were stored, extracted and quantified in Holland using the nanodrop method and samples were delivered to the WTCRF Edinburgh for genotyping.

7.6.4 Control selection – The 1958 Birth Cohort

The British 1958 cohort (also known as the National Child Development Study - http://www.cls.ioe.ac.uk/studies.asp?section=000100020003) includes all births in England, Wales and Scotland, during one week in 1958. From an original sample of over 17,000 births, survivors were followed up at ages 7, 11, 16, 23, 33 and 42 years. Immigrants of the same dates of birth were identified at ages 7, 11 and 16, and followed into adulthood, but adult immigrants (after age 16) have not been included. Data was collected up to age 42 by interviews with parents and cohort members, and at school medical examinations, includes information on growth, health and health

related behaviour, family background, socio-economic circumstances, behavioural, emotional and cognitive development, educational achievement, employment, psychosocial work characteristics, partnership and pregnancy histories. I applied to the clinical oversight committee of the 1958 Birth Cohort, and successfully obtained access to the genotypic data on 4,000 British individuals that were genotyped in 2006 using the Illumina 550K genotyping platform. This control group provided a large group of unselected individuals from the same ethnic background as our cases from London and Cambridge, providing a suitable comparison group to minimise the potential effect of population stratification.

7.6.5 Control selection – Utrecht, Holland

Dr. Roel Ophoff is an associate professor in the Department of Medical Genetics and the Rudolf Magnus Institute of Neuroscience in Utrecht, Holland. I approached him seeking collaboration with the aim of providing a suitable population control group to match the Dutch cases of RRD. As one of his recent projects, he genotyped 750 individuals recruited from outpatient and community centres throughout Nijmegen. These individuals were screened for the absence of psychiatric disease and were genotyped using the Illumina 550k platform in 2007. Participants did not undergo specific ocular examination however all participants were of Dutch origin and lived in the local region providing an appropriate control comparison groups for the Dutch cases of RRD.

7.6.6 Illumina iSelect Platform

Polymorphic variants found to be statistically significant from stage 1 case / control analysis were genotyped in the samples compiled from the developed collaborations.(Table 7.1) The Illumina iSelect platform was used for stage

2 genotyping. The most statistically significant 4,541 SNPs (~1%) from the first stage case control comparison as well as 1,440 SNPs chosen to tag 17 genes (versican/CSPG2, OPTC, LAMC1, LAMB1, LAMA1, KCNJ13, HAS1, HAPLN1, FZD4, FN1, FBN1, COL9A1, COL6A1, COL4A4, COL2A1, COL11A1, COL11A2) that are thought to have a putative biological role in RRD or have been proven to be implicated in syndromic RRD development were selected for genotyping.(Table 7.2)

	CASES (N)		CONTROLS (N)
Scotland	121	1958 Birth Cohort	2,594
Cambridge	470		
London	217		
Nijmegen (Netherlands)	264	Dutch controls	326
Total	1,072		2,920

Table 7.1 demonstrates the number of cases and controls that were available for analysis in stage 2. The 1958 UK birth cohort were used as a control group for the combined samples from Cambridge, London and Scotland.

	Selected SNPs (N)	Genotyped SNPs (N)
GWAS	4541	4076
Candidate genes	1440	1092

Table 7.2 - Summary table highlighting the selected and genotyped SNPs in stage 2. An additional 921 SNPs were not used as they were not genotyped in the new control groups (1958 Birth Cohort , Dutch controls) The final number of SNPs in the stage 2 analysis was 4,235.

CHAPTER 8 – The epidemiology of rhegmatogenous retinal detachment in Scotland- A two-year prospectively recruited population based study

8.1 Sample Size

Over the 2 year study period, a total of 1,202 cases of primary RRD were recruited. Through regular examination of all vitreo-retinal operating logbooks and identification of individual case notes, I am confident that this represents approximately 96% of all operated cases of primary RRD in Scotland during the study period. An additional 42 cases met the inclusion criteria but were missed while in hospital or refused to participate. Including these 42 cases, the annual incidence of RRD was 12.05 per 100,000(95%CI 11.35 - 12.70). Clinical data on these cases was not available and is not included in further analysis.

8.2 Age and Gender Distribution

Table 8.1 shows the baseline characteristics of all cases. The incidence rates are shown in table 8.2. 61.1% of cases were male; 38.9% female. Virtually all cases were of white British ethnicity. The median age at presentation of all cases was 60.2 years (IQR= 49.7 to 67.6 years). The incidence rates varied significantly with age and between genders (Table 8.2); the peak incidence for both sexes was in the 60-69 year age group, with an overall incidence of 33.10 per 100,000(95%CI 29.85-36.65). When examined at 5 year age intervals, a secondary peak in incidence was identified in males aged 15-24 years(4.53 per 100,000; 95%CI 2.53-7.28) and slightly later in females aged 30-39 years at presentation(6.23 per 100,000; 95%CI 3.83-

9.48).(see Figure 8.2) In females this is attributed to a greater predominance of high myopia (>-6 dioptres(D)) in the 30-39 year age group compared to the total case population (33.33% vs 16.63%)(p=0.023). No significant refractive error (>-6D) difference was noted in males aged 15-24 years compared to the case population (14.8% vs 16.6%)(p=0.85), however 38.7% of males in this age group had traumatic RRD compared to 11.8% overall. (p<0.0001)

A significantly higher incidence of all types of RRD was seen in males. This gender difference was also noted in the age standardised incidence (M:F= 1.76:1). Excluding all cases with previous cataract surgery and reported ocular trauma; the male predominance in incidence persisted (M:F-1.4:1). The incidence for males in this group was 9.35 per 100,000 (95%CI 8.55-10.25) and for females was 6.65 per 100,000 (95%CI 5.95-7.35) (p<0.0001)

8.3 Pseudophakic RRD

For descriptive purposes I have divided RRD cases into those that have had previous cataract surgery (pseudophakic or aphakic) and those that have not (phakic). Approximately one in five presenting cases (21.6% - 260/1,202) had previous cataract surgery with intra-ocular lens insertion; if those who have had cataract extraction without an intra-ocular lens inserted are included, this rises to 23.4%. The median time from cataract surgery to presentation with RRD was 3.28 years (IQR- 1.06-7.23 years). Of the pseudophakic cases 46/260 or 17.7% had complicated surgery at the time of cataract removal with vitreous loss with a shorter median time to presentation of 1.38 years (IQR- 0.37-7.23 years). Sixty eight percent

(178/260) of pseudophakic RRD occurred in men; the female pseudophakic RRD group had a higher proportion of complicated cataract surgery (23.1%-19/82 vs 15.1%-27/178).

8.4 Refractive error

Spherical equivalent refractive error(SER) measurements were calculated as dioptres (D). To avoid any potential inaccuracies between measured SER in phakic and pseudophakic/aphakic cases (which in some cases may have been recorded after cataract surgery), only the SER of the 920 phakic patients are included for further analysis.(Table 8.1) However, table 8.3 is a comparison between the refractive error classifications of phakic and combined pseudophakic/aphakic cases demonstrating similar proportions noted. The majority of cases (53.2%) were myopic with an SER of ≥-1D and 18.1% of all cases were highly myopic with an SER of >-6D. Figure 8.1 demonstrates the age distribution of all cases as a function of SER. Under the age of 50, 82.14% (161/196) of phakic cases exhibit myopia >-1D. With increasing age this trend diminishes, with an increasing proportion of emmetropic and hypermetropic individuals affected. (Figure 8.1) There was no difference in the gender distribution of RRD associated with myopia(p=0.217)(Figure 8.3).

8.5 Laterality

The right eye was affected significantly more frequently than the left eye (54.9% vs 43.4%)(p<0.0001) in both men and women. 1.5% (18/1202) of cases described unilateral symptoms, but clinical examination revealed RRD to be present in both eyes. 70/1202 or 5.8% had a previous RRD in the

fellow eye outside the study period, and 8/1202 or 0.67% suffered a consecutive fellow eye RRD during the study period.

8.6 Indices of Deprivation

Postcode data was available for matching to SIMD rank for 1,178 cases. Figure 8.4 shows the age standardised annual incidence of primary RRD per quintile of ranked deprivation for both genders. The age standardised incidence of RRD rose from 9.15 to 13.5 per 100,000 between the most deprived and the least deprived quintile, with a strong association across quintiles of increasing affluence (χ^2 for trend = 22.48 $p=2.11x10^{-6}$). This was greater for men (χ^2 for trend = 18.74 $p=1.49x10^{-5}$) than women (χ^2 for trend = 4.08 p=0.043). A similar trend was observed across the component domains making up the SIMD score, with a higher incidence in the most affluent quintiles. A significant difference was found between the domains of income, employment, health, education and housing. ($p<0.0001$) The strongest association was found in the domain of education (χ^2 trend=40.22;$p=2.27x10^{-10}$). Geographic access to essential services demonstrated the opposite trend (χ^2 trend = 8.29; p=0.004). (See Figure 8.6)

Attachment of the macula at presentation, the most important prognostic factor for final visual outcome, showed a marked variation across quintiles of deprivation. (Table 8.4) 65.13% of cases in the most deprived quintile presented with a detached or bisected macula at diagnosis compared to 50.86% in the most affluent quintile. (χ^2 for trend = 6.83, p-value = 0.0089)

The extent of detachment may be an indicator of chronicity of RRD and this also demonstrated significant variation across quintiles of deprivation.(Table

8.5) One quadrant of detachment was more frequent in least deprived quintile compared to the most deprived (29% vs 18%, χ^2 for trend = 9.69, p-value = 0.0018). Total RRD (four quadrants of detachment) was much more frequent in the most deprived quintile compared to the most affluent. (13% vs 4%, χ^2 for trend = 14.17, p-value = 0.0001)

A higher proportion of cases from the most deprived quintile had previous cataract surgery compared to the least deprived (28.39% vs 18.79%; χ^2 = 8.74, p-value = 0.003). There were no significant differences between quintiles of deprivation in the proportions of significant ocular trauma. (Table 8.6 and 8.7)

Refractive error and SIMD ranking was available for 90.4% (832/920) of phakic cases. Figure 8.5 illustrates the proportion of low myopia (≥-1 to ≤-6D) and high myopia (>-6D) across quintiles of deprivation for these cases. A significant trend was noted for low myopia (≥-1 to ≤-6D) (χ^2 for trend = 7.85, p-value = 0.005), with 32.4% of cases in the most affluent quartile having low myopia compared with 22.4% in the most deprived. No such trend was observed for high myopia (>-6D). (16.4% in the most affluent quartile versus 11.7% in the most deprived; χ^2 for trend = 1.34, p-value = 0.2462) Examining all cases with spherical equivalent refractive error >-1D a significant trend was noted (χ^2 for trend**=** 11.19, p-value = 0.00081), with 48.8% of cases in the most affluent quartile compared with 34.1% in the most deprived.

8.7 Conclusions

I have undertaken one of the largest prospectively recruited epidemiology studies of primary RRD and found the annual incidence in a population of 5.1 million to be 12.05 per 100,000 of population. In the U.K., this equates to approximately 7,300 incident cases of primary RRD annually.

There have been several studies over the last 40 years whose principal aim was to estimate the incidence of primary RRD, however a wide range of results have been reported.(Wong *et al.* 1999; Tornquist *et al.* 1987a; Algvere *et al.* 1999; Haimann *et al.* 1982a; Limeira-Soares *et al.* 2007; Rowe *et al.* 1999; Laatikainen *et al.* 1985; Ivanisevic *et al.* 2000; Sasaki *et al.* 1995; Polkinghorne & Craig 2004b; Mowatt *et al.* 2003; Zou *et al.* 2002; Wilkes *et al.* 1982) Only one study from Beijing, China,(Li 2003) examined a population at risk comparable to ours in size and estimated a lower annual incidence of 7.98 per 100,000 (95%CI-7.3-8.67). This difference may be attributable in part to the age distribution of the study population. In Beijing, 14% of the population were over 60 years, in Scotland 22.6% were above 60 years. Examining previous reports with a sample size of over 500 cases, a minimum recruitment period of 1 year and pre-defined case inclusion criteria the estimated annual incidence varied nearly two-fold, between 7.98 per 100,000 to 14 per 100,000.(Li 2003; Tornquist *et al.* 1987a; Wong *et al.* 1999; Algvere *et al.* 1999) Analysing previous reports from European countries only, I note a similar variation in annual incidence between 6.9 per 100,000 to 14 per 100,000 of population.(Laatikainen *et al.* 1985; Algvere *et al.* 1999) There are several possible reasons for the noted variation: the methodology used in previous studies has differed considerably[18] ;there were no clearly defined inclusion criteria across studies making accurate comparison problematic. RRD incidence varies with age, gender, affluence

and prevalence of both myopia and pseudophakia, thus characteristics of the underlying study population will influence the reported incidence. Finally, changing treatment modalities for RRD and a move toward more daycase surgery and out-patient procedures(Sodhi *et al.* 2008) may influence case recording, making comparison of rates between countries and different time periods difficult.

There is a large difference in the annual incidence of RRD between men and women. (M:F=1.68:1) This increased incidence was significant and persisted in the age standardised incidence ratios (M:F=1.76:1) and was not affected by excluding trauma and previous cataract surgery (M:F= 1.40:1). Within the pseudophakic and aphakic group, the overrepresentation of males was even more marked (M:F=2.3:1), despite a higher rate of cataract surgery in women in the U.K.(Keenan *et al.* 2007b) Most previous studies show a higher incidence in males,(Mowatt *et al.* 2003; Ivanisevic *et al.* 2000; Rosman *et al.* 2001; Polkinghorne & Craig 2004b; Limeira-Soares *et al.* 2007)(M:F=1.3:1 to 2.3:1); a minority find that females predominate in the phakic non-traumatic group.(M:F= 1:1.16 to 1:1.4)(Tornquist *et al.* 1987a; Sasaki *et al.* 1995) A meta-estimate of previous studies reporting the gender distribution in RRD incidence indicate a male proportionality of between 52-59%($p<0.0001$)(Figure 8.7). There may be several reasons behind this gender imbalance in RRD incidence.

Long term cohort studies in Taiwan have demonstrated that the risk of RRD after cataract surgery is higher in males. The increased risk of RRD in patients with myopia and a history of RRD was seen to be significant in males only, up to 4 years after cataract surgery.(Sheu *et al.* 2007; Sheu *et al.* 2009)

In this study males with RRD had a significantly longer axial length in their fellow eye than females (mean;standard deviation); males [N=330,

25.05mm(1.65)], females [N = 24.39mm (1.64), T=4.51; p<0.001] The Beaver Dam Eye Study(Lee *et al.* 2009) also reported a greater axial length in white males compared to females [males 23.92mm (1.10); females 23.51mm (1.17); p<0.001)], as do other large population-based studies. An increase in axial length is significantly associated with an increased risk of RRD(Tuft *et al.* 2006).

Gender differences in the anatomy of the vitreoretinal base may also contribute to differences in the incidence of RRD. In a study of donor eyes, Wang et al(Wang *et al.* 2003) reported a posterior migration of the posterior border of the vitreous base in males. This may predispose males to retinal breaks after posterior vitreous detachment, either from greater dynamic vitreoretinal traction, and/or an increase in vitreoretinal irregularities of the posterior border. Hence, both the longer axial length in males and differences in basal vitreoretinal adhesion may contribute to the higher incidence of RRD. Furthermore, perhaps due to lifestyle differences, males tend to under-report 'lesser' trauma that may contribute to RRD risk.

The age distribution of our cases indicates a peak in both genders in the 60-69 year age group. This is widely supported in previous studies. (Wong *et al.* 1999; Tornquist *et al.* 1987a; Algvere *et al.* 1999; Haimann *et al.* 1982a; Limeira-Soares *et al.* 2007; Rowe *et al.* 1999; Laatikainen *et al.* 1985; Ivanisevic *et al.* 2000; Sasaki *et al.* 1995; Polkinghorne & Craig 2004b; Mowatt *et al.* 2003; Zou *et al.* 2002; Wilkes *et al.* 1982)

The right eye was involved significantly more frequently than the left eye (1.26:1), the majority of previous studies support this finding- (ratios ranging between 1.09:1 to 1.36:1)(Tornquist *et al.* 1987a; Haimann *et al.* 1982a; Laatikainen *et al.* 1985; Li 2003; Rowe *et al.* 1999)Despite excluding all cases with previous cataract surgery and reported trauma, I found a right eye to left eye ratio of 1.18:1. (p=0.001) A meta-estimate of previous

studies reporting this indicates a right eye proportionality of 53.5-56.7%($p<0.0001$)(Figure 8.7). The explanation for the greater incidence in the right eye remains unknown. However, right eyes are more frequently dominant, and dominant eyes are frequently more myopic(Cheng *et al.* 2004). Nonetheless, in this series of RRD cases, there was no difference in the axial length of right and left fellow eyes [right eyes, N = 243, 24.87mm(1.64); left eyes, N = 285 24.75mm(1.71); T=0.82; p=0.42]. Similarly, in other population studies, there was no difference in right and left axial lengths(Lee *et al.* 2009)

Socioeconomic status can affect the incidence of many diseases and the association between deprivation and visual impairment is well known.(Tielsch *et al.* 1991) Unexpectedly, I found a disparity in the incidence of RRD between socio-economic groups, with an association between affluence and RRD. This trend was a significant finding in both genders but was much more marked in men. The trend was significant in five of seven socio-economic demographic domains that determine the overall deprivation score and the strongest association was found in the domain of educational achievement (χ^2 trend = 40.21; p-value = 2.3×10^{-10}). In addition to the association with affluence, the characteristics of RRD at presentation showed dramatic variation between quintiles of deprivation. RRD cases from the most deprived quintile more frequently presented with a total RRD (13% vs 4%) and more frequently presented when the macula was detached (65% vs 51%) when compared to the least deprived quintile. These findings indicate that cases from more deprived areas tend to present later and with more extensive detachments. This has important consequences for final visual prognosis.

I explored several possible explanations for the association between RRD and affluence. The incidence of RRD increases with age to a peak in the

sixth decade. Age specific mortality rates may differ between the most deprived and least deprived quintiles so that fewer people from deprived areas live long enough to be at greatest risk of retinal detachment. Similarly, elderly individuals may have accrued more wealth over many years and a larger proportion of elderly individuals may live in affluent areas. However there was a significant increase in the age specific incidence of RRD across quintiles of deprivation in the age groups comprising the highest natural incidence of RRD (age groups 50-59 and 60-69), suggesting that the influence of age was not the primary factor behind the association with affluence . (see Table 8.8)

Trauma may influence the incidence of RRD however the proportion of traumatic cases was equal across quintiles. Previous cataract surgery, a known risk factor for RRD did not significantly influence the association with affluence as more pseudophakic RRD cases were present in the more deprived quintiles.

This study has only recorded patients on presentation to hospital, and it is possible that patients from areas of greater deprivation have poorer access to healthcare services and have been excluded from the study. However, based on the SIMD classification of our cases, those from the most deprived areas ranked higher than those from the least deprived areas in the geographic access to services domain. This suggests that access to essential services was not a limiting factor in presentation to hospital.

Myopia is a significant risk factor for RRD and has been associated with higher educational achievement and IQ, and thus, perhaps, higher income and socio-economic status. (The Eye Disease Case-Control Study Group 1993; Williams *et al.* 2008; Saw *et al.* 2004) It is interesting to note that of the SIMD rank determinants, the strongest association was found between RRD and educational achievement. Detachments in the most

affluent quintiles were more likely to be associated with myopia than those in the most deprived quintiles. This increased proportion of myopic RRD cases in more affluent areas is an important factor that partly explains the rise in RRD incidence between the most and least deprived quintiles. Although myopia is an important factor in the observed association between RRD and affluence, I cannot exclude other as yet unidentified risk factors associated with socio-economic status which may underlie this observation.

In summary, I have prospectively estimated the overall incidence of primary RRD in Scotland to be 12.05 per 100,000 population. Males are affected more than females in all age groups and all types of RRD. Over 50% of all phakic cases are myopic. One in five cases with RRD have had previous cataract surgery. RRD incidence and the proportion of myopic RRD are significantly associated with affluence, however RRD cases from more deprived datazones frequently present with a more extensive area of detachment.

Baseline Characteristics	
Year of Diagnosis	**Number of patients (%)**
2007/2008	594(49.4%)
2008/2009	608(50.5%)
Sex	
Male	735(61.1%)
Female	467(38.9%)
Ethnicity	
White British	1,176(97.9%)
Pakistani	7(0.6%)
Chinese	6(0.5%)
Indian	4(0.3%)
Black	2(0.2%)
Other	6(0.5%)
Age Group	
0-9	2(0.2%)
10-19	27(2.2%)
20-29	40(3.3%)
30-39	90(7.4%)
40-49	145(12.1%)
50-59	292(24.3%)
60-69	371(30.9%)
70-79	179(14.9%)
80+	56(4.7%)
Affected Eye	
Right	661(54.9%)
Left	522(43.4%)
Both(simultaneous)	18(1.5%)
Phakic Status	
Phakic	920(76.5%)
Pseudophakic	260(21.6%)
Aphakic	22(1.8%)
Spherical Equivalent Refractive Error (Dioptre)*	
≥+6 D	7(0.8%)
>+1D to <+6D	79(8.6%)
≥-1D to ≤+1D	269(29.2%)
>-1 D to <-6 D	323(35.1%)
≥-6 D	166(18.1%)
Not Known	76(8.2%)

*Spherical equivalent refractive error (SER) of all 920 phakic cases of RRD.

Table 8.1 – Baseline characteristics of the study population

	Annual Incidence		
	Incidence/100,000 (95%CI)		
Overall Annual Incidence	11.65 (11-12.30)	12.05(11.35-12.70)*	
Sex	**Male**	**Female**	**P-value****
Overall Incidence	14.70(13.60 - 15.80)	8.75(8-9.60)	<0.0001
Age Group			
0-9	0.35(0.10-1.30)	-	-
10-19	2.65(1.50-4.25)	1.65(0.80-3)	0.3
20-29	3.30(2.10-4.90)	2.50(1.45-4)	0.49
30-39	7.30(5.35-9.70)	6.20(4.45-8.30)	0.48
40-49	11.90(9.55-14.60)	6.55(4.90-8.55)	<0.0001
50-59	28.90(24.95-33.33)	14.60(11.90-17.75)	<0.0001
60-69	45.85(40.30-50)	21.40(17.80-25.50)	<0.0001
70-79	27.10(21.85-33.20)	19.85(15.90-24.50)	0.04
80+	16.70(10.8-24.65)	10.70(7.25 - 15.20)	0.13
Age standardised Incidence	13.09(11.23-14.95)	7.41(6.43- 8.39)	<0.0001
Traumatic RRD	2(1.60-2.40)	0.5(0.35-0.75)	<0.0001
Non traumatic RRD	12.70(11.75 - 13.75)	8.25(7.50-9.05)	<0.0001

* Overall incidence of all eligible cases
**P-value for male and female incidence comparison

Table 8.2 – Annual incidence of primary RRD based on all diagnosed cases in Scotland over a 2 year period

	Phakia N=920	**Combined Pseudophakia (N=260) and Aphakia (N=22)**
Median age at presentation (IQR)	58.71(48.02-65.77)	63.86(55.44-72.53)
Spherical Equivalent Refractive Error (Dioptre)*		
≥+6D	7(0.76%)	7(2.5%)
>+1 to <+6D	79(8.58%)	23(8.2%)
≥+1 to ≤-1D	269(29.23%)	65(23%)
>-1 to <-6D	323(35.10%)	92(32.6%)
≥-6D	166(18.04%)	31(11%)
Not Known	76(8.2%)	64(22.7%)

Table 8.3 – Comparison of refractive error between phakic and combined pseudophakic/aphakic individuals. The proportions of myopia were similar between both groups, however the combined pseudophakic/aphakic group had a higher proportion of unknown refractive errors.

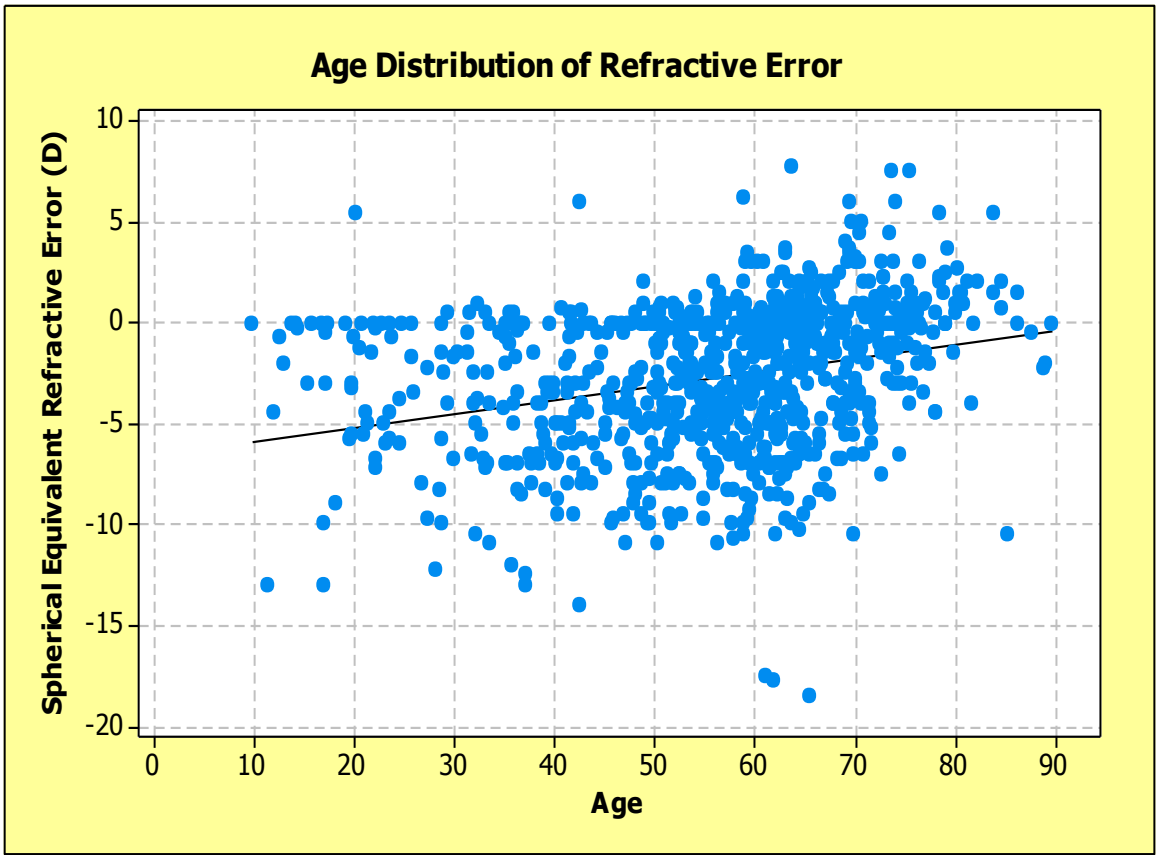


Figure 8.1 – Scatterplot and trendline of age and spherical equivalent refractive error for all 920 phakic cases of primary RRD.

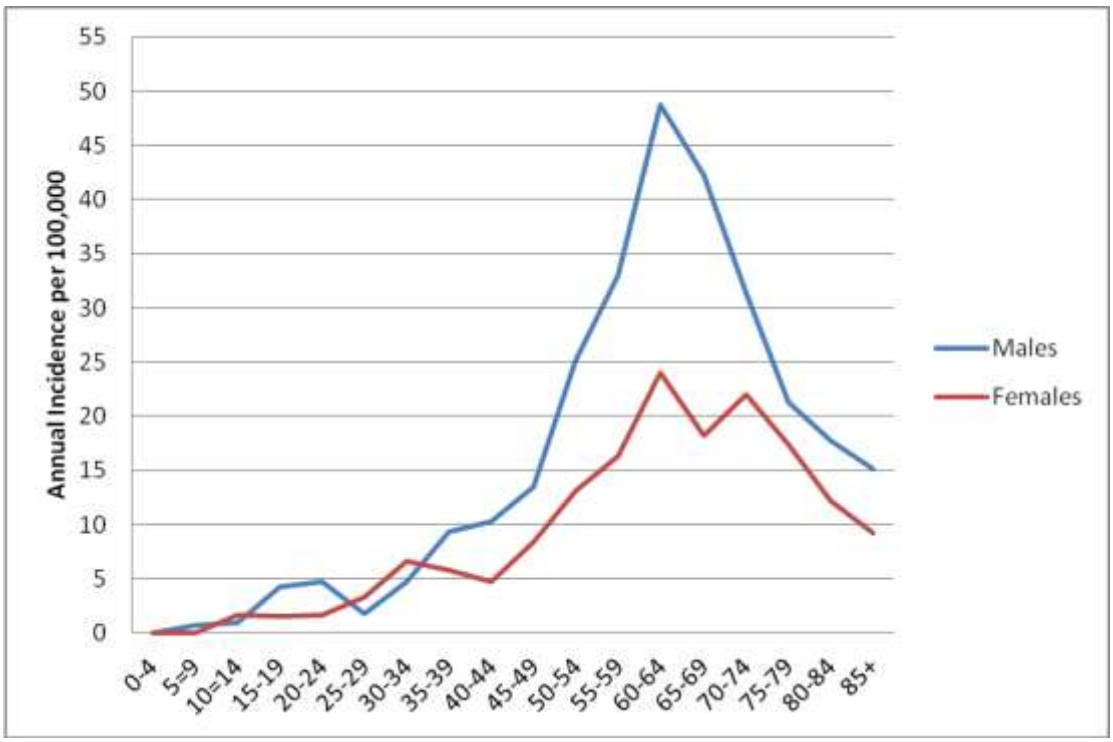


Figure 8.2 – Annual RRD incidence by gender and 5 year age group in Scotland

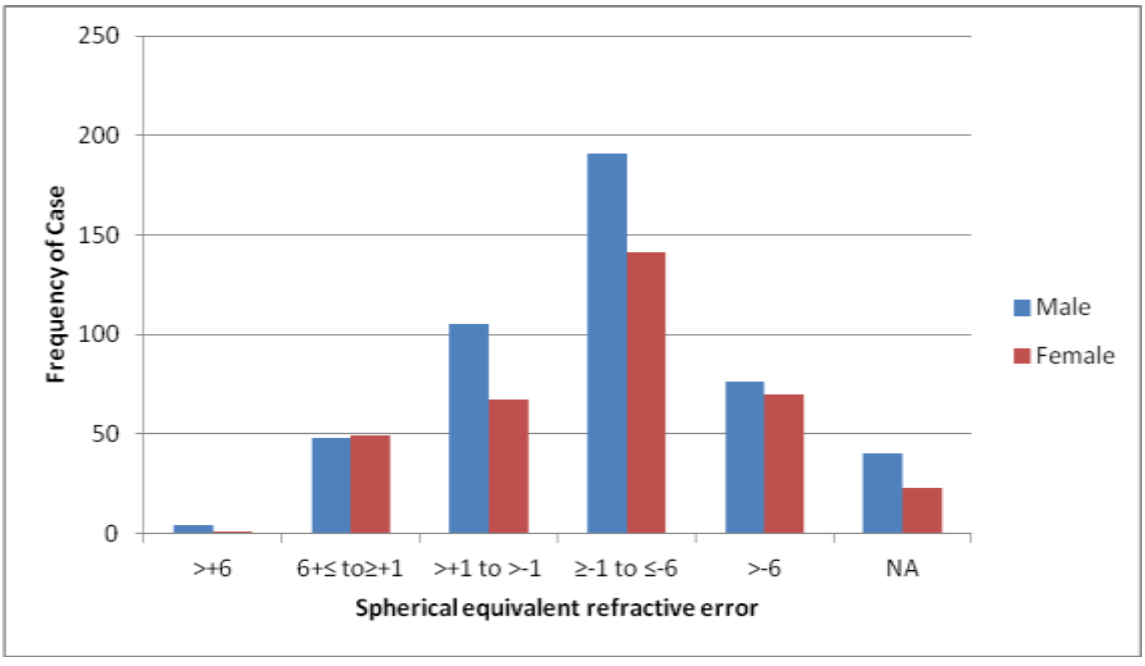


Figure 8.3 – The gender distribution of refractive error among all phakic cases of RRD. (χ^2 = 7.037, p-value = 0.2179)

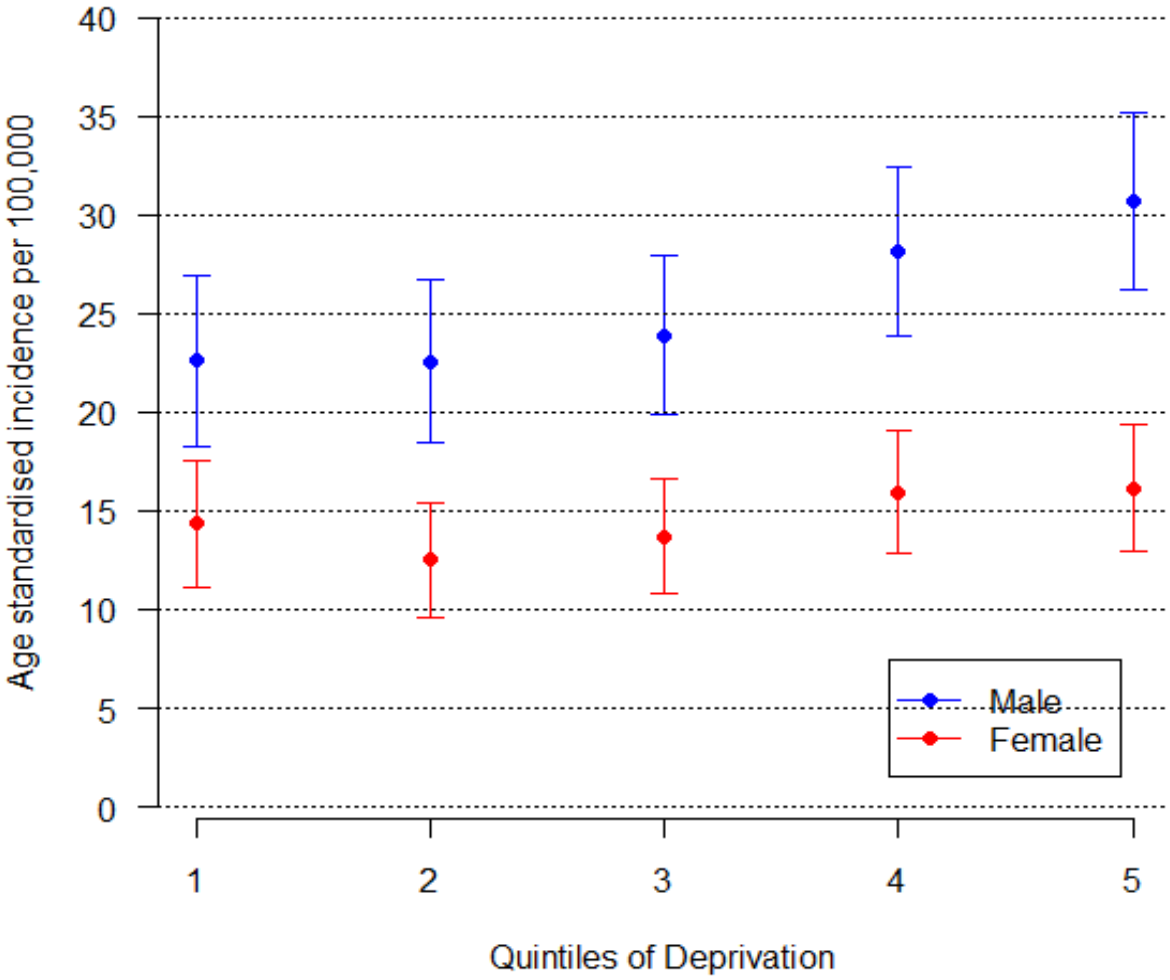


Figure 8.4 – Age standardised incidence and 95% confidence interval of primary RRD by quintiles of deprivation in males (blue) and females (red). (χ^2 for trend males = 18.74 p=1.49x10^{-5}; χ^2 for trend females = 4.08 p=0.043) (1=Most deprived quintile; 5=Least deprived quintile)

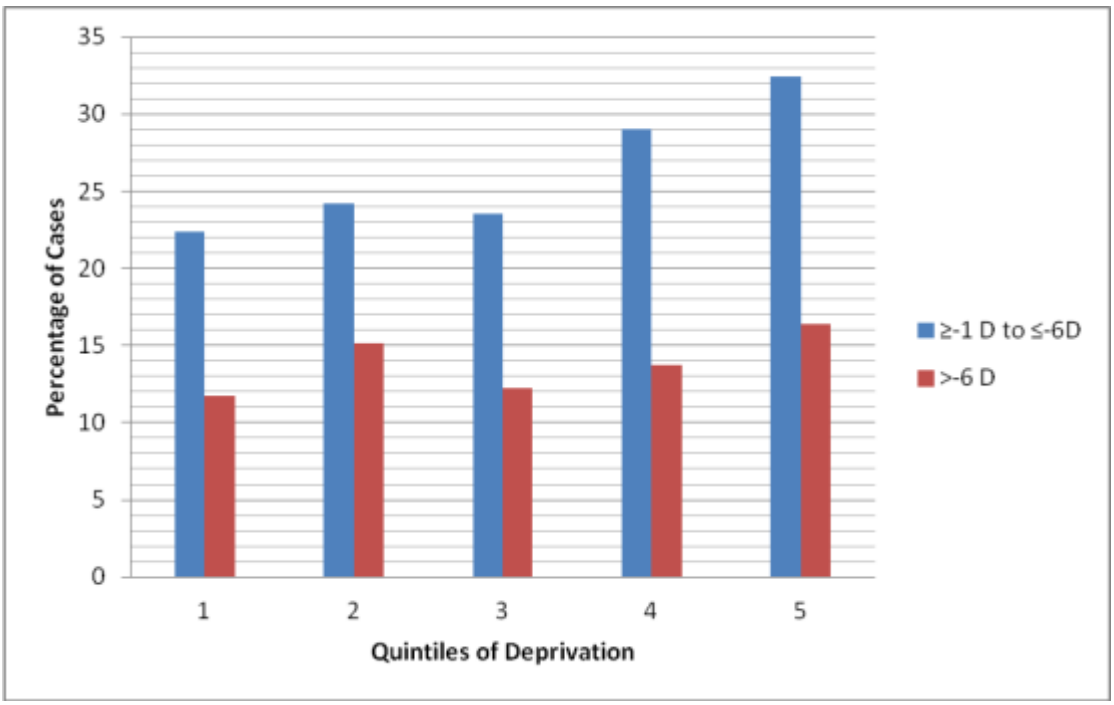
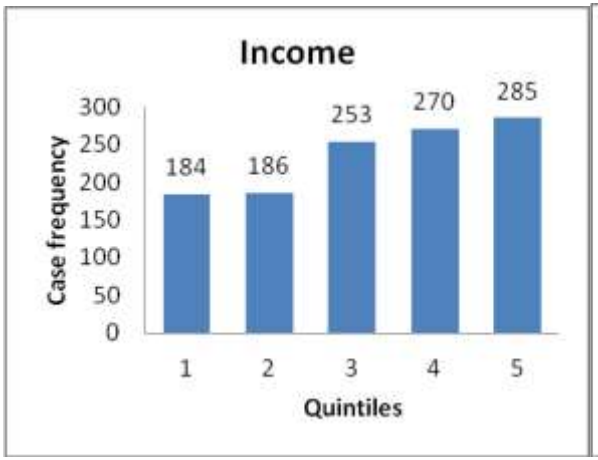
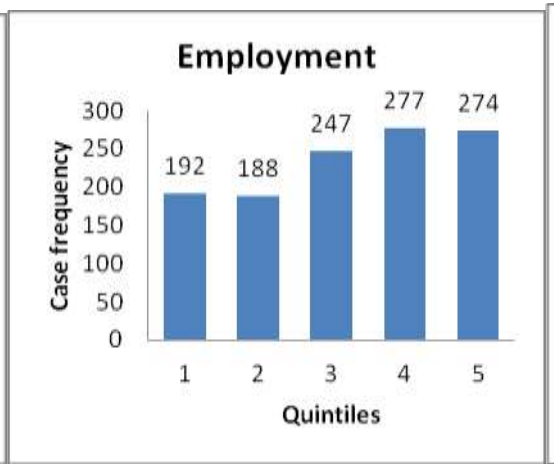


Figure 8.5 – Bar plot demonstrating the distribution of low and high myopia across quintiles of deprivation for 90.4% (832/920) of all phakic cases. (Low myopia (≥-1D to ≤-6D): χ^2 for trend = 7.85, p-value = 0.005; High myopia (>-6D) χ^2 for trend = 1.34, p-value = 0.2462; All spherical equivalent refractive errors >-1D: χ^2 for trend**=** 11.1987, p-value = 0.00081) (1=Most deprived quintile; 5=Least deprived quintile)


Income
Case frequency
300
250
200
150
100
50
0
184
186
253
270
285
1
2
3
4
5
Quintiles


Employment
Case frequency
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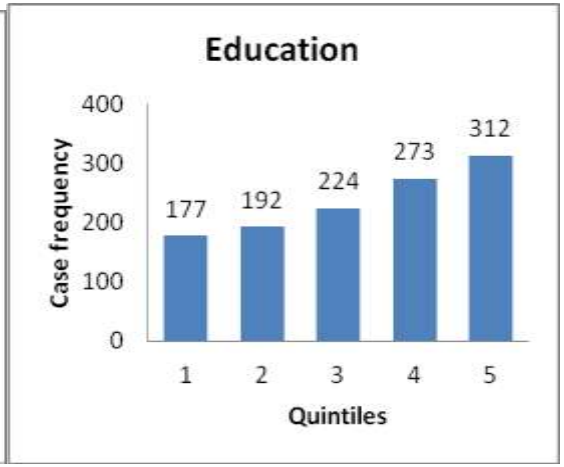
200
150
100
50
0
192
188
247
277
274
1
2
3
4
5
Quintiles


Education
Case frequency
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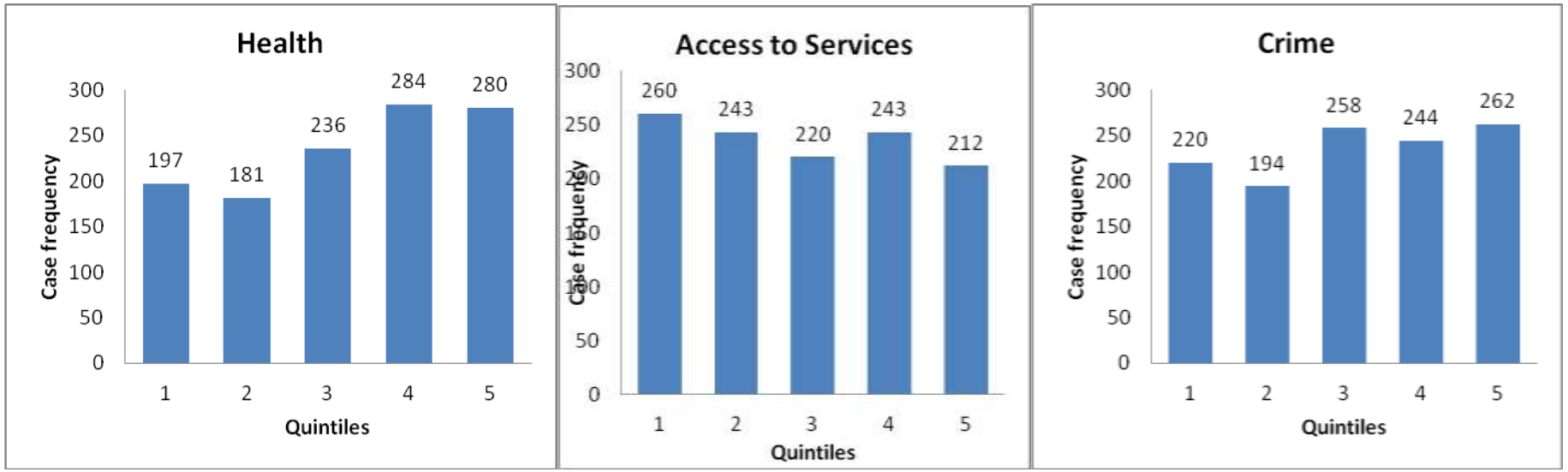
300
200
100
0
177
192
224
273
312
1
2
3
4
5
Quintiles


Health
Case frequency
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250
200
150
100
50
0
197
181
236
284
280
1
2
3
4
5
Quintiles
Access to Services
Case frequency
300
250
50
0
260
243
220
243
212
1
2
3
4
5
Quintiles
Crime
Case frequency
300
250
200
150
100
50
0
220
194
258
244
262
1
2
3
4
5
Quintiles

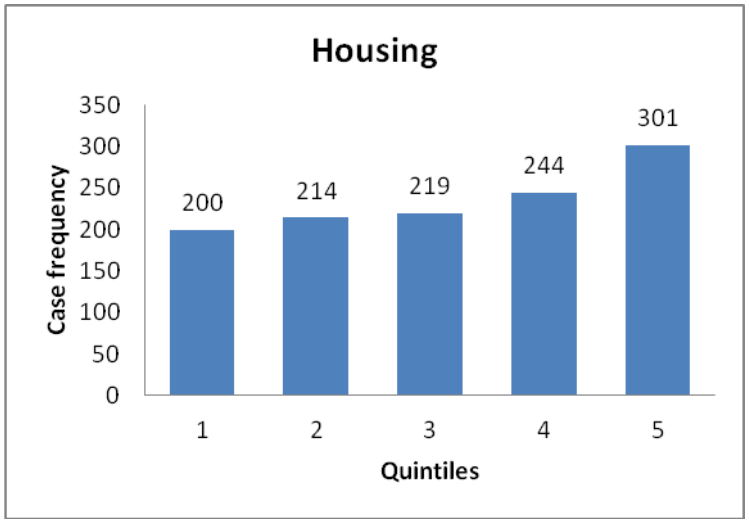


Figure 8.6abcdefg – Histograms showing the Scottish Index of Multiple Deprivation scores, by quintiles, for 1,178 incident cases of primary RRD in Scotland. Figure 8.6a-g represents the frequency of cases in each of seven domains comprising the overall SIMD score ordered by domain weight. Significant trend associations with affluence were found in five of seven domains. In the geographic access to services domain, the opposite association, between deprivation and better geographic access was noted. The association between RRD frequency and affluence was strongest in the education and income domain. **Income (a)**: χ^2 for trend = 25.0079, p-value = 5.71×10^{-7} **Employment (b):** χ^2 for trend = 18.6629, p-value = 1.56×10^{-5} **Education (c):** χ^2 for trend = 40.2163, p-value = 2.273×10^{-10} **Health (d):** χ^2 for trend = 21.6235, p-value = 3.318×10^{-6} **Access to Services (e):** χ^2 for trend = 8.29, p-value = 0.003986 **Crime (f):** χ^2 for trend = 3.4793, p-value = 0.06214 **Housing (g):** χ^2 for trend = 15.1077, p-value = 0.0001015

Quintile	Macula On	% Macula On	Macula Off/Bisected	% Macula Off/Bisected	Total
1	65	34.86	123	65.13	188
2	83	41.50	115	58.49	198
3	100	43.66	129	56.33	229
4	110	39.82	166	60.17	276
5	141	49.13	146	50.86	287

Table 8.4 – The proportion of macula affected RRD in quintiles of deprivation. A higher proportion of macula affected cases were noted in the most deprived quintile, with a significant decreasing trend across higher ranked quintiles. (χ^2 for trend = 6.8364, p-value = 0.008932) (1=Most deprived quintile; 5=Least deprived quintile)

Quintile	One Quadrant	% One Quadrant	Two Quadrants	%Two Quadrants	Three Quadrants	%Three Quadrants	Four Quadrants	%Four Quadrants	Not Known	%Not Known	Total
1	34	18.08	69	36.89	46	24.55	25	13.16	14	7.29	188
2	32	16.04	95	48.07	34	17.20	23	11.34	14	7.32	198
3	48	20.87	100	43.51	48	20.82	18	7.826	16	6.94	230
4	54	19.61	132	47.81	55	19.96	18	6.504	17	6.10	276
5	83	29.00	119	41.36	48	16.71	14	4.87	23	8.03	287

Table 8.5 – The extent of RRD in quadrants of detachment ordered by quintiles of deprivation. A higher proportion of one quadrant detachments was noted in the least deprived quintiles. By contrast, a higher proportion of total detachment was noted in the most deprived quintiles with a significant decreasing trend with higher socio-economic ranking. (1=Most deprived quintile; 5=Least deprived quintile)

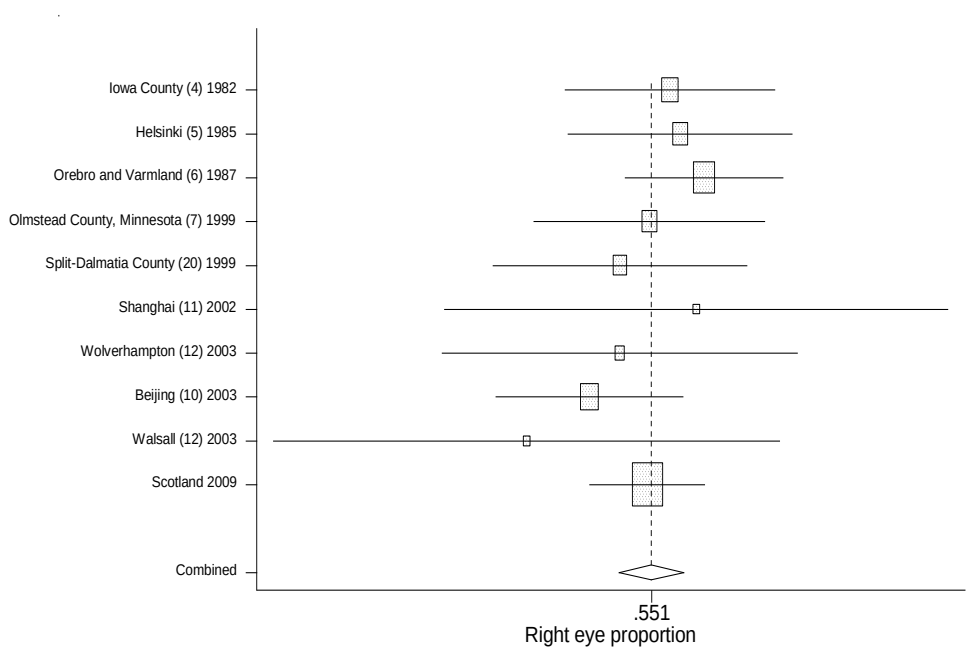
One quadrant χ^2 for trend = 9.6918, p-value = 0.001851
Two Quadrants χ^2 for trend = 0.4757, p-value = 0.4904
Three Quadrants χ^2 for trend = 2.3238, p-value = 0.1274
Four Quadrants χ^2 for trend = 14.171, p-value = 0.0001669
Not Known χ^2 for trend = 0.0117, p-value = 0.914

Quintile	Trauma	% Trauma	Total
1	26	13.83	188
2	17	8.59	198
3	24	10.48	229
4	26	9.42	276
5	29	10.1	287

Table 8.6- The distribution of traumatic RRD across quintiles of deprivation. (1=Most deprived quintile; 5=Least deprived quintile) χ^2 for trend = 0.8607, p-value = 0.3536

Table 8.7- The distribution of phakic and pseudophakic or aphakic cases across quintiles of deprivation. No significant difference was found in proportions of pseudophakia or aphakia across quintiles of deprivation.

Quintile	Phakic	% Phakic	Pseudophakic/Aphakic	% Pseudophakic/Aphakic	Not Known	Total
1	135	71.60	53	28.39	0	188
2	145	73.37	53	26.62	0	198
3	171	74.65	58	25.34	0	229
4	220	79.65	55	19.96	1	276
5	231	80.50	54	18.79	2	287



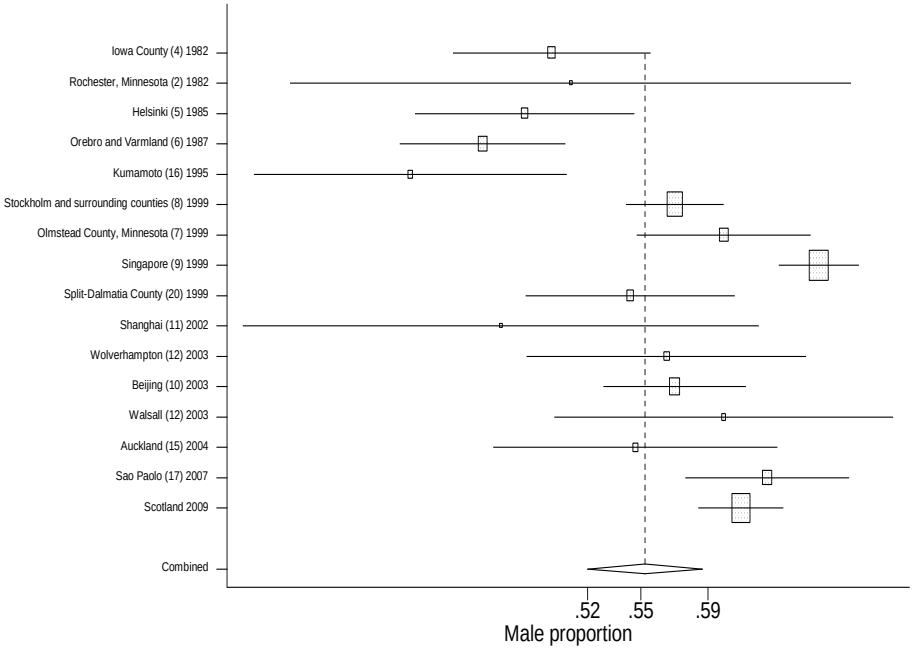


Figure 8.7 – Forest plot demonstrating the meta-estimate and 95% confidence interval for the reported proportion of males affected and the reported proportion of right eye affected in previous population based epidemiology studies. Males were affected more frequently 52%(95%CI= 52-59%, p-value <0.001). The right eye was affected more frequently 55.1%(95%CI=53.5-56.7%, p-value <0.001)

Quintile	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80+
1	0(0-0)	0.54(0.10-1.8)	0.46(0.07-1.54)	1.4(0.46-3.48)	2.1(0.92-4.18)	3.69(1.78-6.67)	5.17(2.81-8.97)	3.83(1.53-7.93)	1.85(0.21-7)
2	0.09(0.002-0.53)	0.42(0.05-1.67)	0.62(0.11-2.10)	1.58(0.56-3.61)	1.67(0.68-3.55)	2.76(1.29-5.26)	5.27(2.93-8.79)	4.4(2.02-8.37)	1.73(0.21-6.28)
3	0(0-0)	0.16(0.005-0.93)	0.69(0.15-2.27)	1.32(0.41-3.20)	1.37(0.48-3.09)	4.32(2.42-7.14)	5.6(3.24-9.05)	4.95(2.42-9.09)	3.1(0.82-8.42)
4	0.08(0.002-0.48)	0.8(0.16-2.54)	0.55(0.075-2.17)	1.24(0.375-3.075)	1.83(0.785-3.6)	4.13(2.38-6.25)	8.02(4.43-11.86)	5.24(2.57-9.51)	1.50(0.19-5.88)
5	0(0-0)	0.23(0.01-0.97)	0.59(0.17-1.47)	1.02(0.26-2.85)	2.06(0.96-3.91)	5.81(3.64-8.80)	7.74(4.91-11.66)	4.11(1.79-8.19)	4.56(1.52-10.68)

Table 8.8 – The age specific incidence and associated 95% confidence interval of RRD by quintile of socioeconomic deprivation. (1=Most deprived quintile; 5=Least deprived quintile) A significant increase was found in RRD incidence in age groups 50-59, 60-69 and 80+ years across quintiles.

Age group 0-9 χ^2 for trend = 0.001, p-value = 0.9696
Age group 10-19 χ^2 for trend = 0.260, p-value = 0.61
Age group 20-29 χ^2 for trend = 0.144, p-value = 0.7041
Age group 30-39 χ^2 for trend = 1.604, p-value = 0.2053
Age group 40-49 χ^2 for trend = 0.045, p-value = 0.8311
Age group 50-59 χ^2 for trend = 10.64, p-value = 0.001103
Age group 60-69 χ^2 for trend = 10.99, p-value = 0.0009124
Age group 70-79 χ^2 for trend = 0.331, p-value = 0.5651
Age group 80+ χ^2 for trend = 4.793, p-value = 0.02850

CHAPTER 9 - Clinical characteristics in the Scottish rhegmatogenous retinal detachment study I – Predisposing pathology

9.1 Introduction

Rhegmatogenous retinal detachment (RRD) is a common sight-threatening condition initiated by a full thickness break in peripheral retina. Predisposing associations of RRD, such as high myopia, previous cataract surgery, direct ocular trauma and peripheral retinal degenerations are well established(The Eye Disease Case-Control Study Group 1993; Tielsch *et al.* 1996), however, the incidence of these features and their relative contribution to RRD occurrence in populations is not well documented. Similarly, the occurrence of hereditary vitreo-retinal degenerations in RRD populations is uncertain. As part of the Scottish Retinal Detachment Study, I analysed the predisposing characteristics of 1,202 incident cases of primary RRD presenting to all vitreoretinal services in Scotland between 2007 and 2009 and present the epidemiological features by vitreous status and type of retinal break, and examine the prevalence of predisposing factors such as myopia, trauma, peripheral retinal degeneration and cataract surgery. Associated features in the fellow eyes of RRD cases are described, as are the epidemiological characteristics of familial and syndromic RRD.

9.2 Results

A total of 1,202 incident cases of primary RRD were recruited in Scotland over the 2 year study period. An additional 42 cases were identified but did not participate in the study and are not included in this analysis. In 1,130 cases there was detailed data on the clinical features of RRD. All cases were classified by the presence or absence of a posterior vitreous detachment and

by the type of retinal break causing the detachment. In total, PVD was present in 87.6% (990/1130) of cases, with single or multiple HST RRD accounting for 98.5% (975/990) of this group and giant retinal tear RRD accounting for 1.5% (15/990). PVD was absent in 12.4% (140/1130) of cases and these comprised round hole RRD in 40% (56/140), retinal dialysis in 47.8%(67/140) and retinoschisis RRD in 12.1%(17/140). The annual incidence of HST RRD was 9.45 (95%CI= 4.02-8.85) per 100,000, of GRT RRD was 0.15 (95%CI = 0.1-0.25) per 100,000, of RH RRD was 0.55 (95%CI = 0.4-0.7) per 100,000, of retinal dialysis was 0.65 (95%CI = 0.5-0.8) per 100,000, and of retinoschisis RRD was 0.15 (95%CI = 0.1-0.25). Continuous data was analysed using a two-sample T-test and differences in categorical data was analysed using the Z-test of proportionality.

9.2.1 Age and gender Distribution

Table 9.1 highlights the age and gender distribution of the subtypes of RRD. Males predominated in both PVD associated RRD (61.7%- 95%CI=58.6-64.7%;M:F ratio= 1.6:1; Z=10.73;p<0.0001) and in RRD without PVD(60%- 95%CI=51.4-68.1%; M:F ratio= 1.5:1; Z=3.42;p=0.001). Cases of RRD without a PVD were significantly younger than cases associated with a PVD. The mean(SD) age of the group without PVD was 43.3(18.9) years versus 59.6(13.3) in the PVD present group. (T=9.89; p<0.001)

9.2.2 Affected Eye

The right eye was affected more frequently than the left eye (55.2%-95%CI=52-58.3%;RE:LE ratio 1.23:1; Z=4.7;p<0.0001) in RRD associated with PVD. There was no difference was seen in the laterality of RRD in the absence of PVD. (Right eye affected in 49.3%- 95%CI=40.7-57.8%;p=0.9)

In all subtypes of RRD a small proportion of cases presented with simultaneous bilateral RRD.(see Table 9.1)

9.2.3 Refractive Error

There were marked differences in refractive error depending on the type of RRD.(Table 9.1) Overall, GRT RRD and RH RRD had the largest proportion of high myopia (26-27%). Approximately 60% of all retinal dialysis cases were emmetropic. A significant difference was noted between RRD associated with a PVD and round hole RRD without PVD. (PVD RRD group: mean(SD) spherical equivalent refraction (D) = -2.63D(3.65); RH RRD group: mean(SD) spherical equivalent refraction(D) = -4.09D(4.55); T=2.21;p=0.032) Overall in the series, high myopia (>-6D) comprised 16.8% (190/1130) of cases. Excluding the eighteen aphakic cases, there was no significant difference in the (fellow eye) axial length and spherical equivalent refractive error between phakic and pseudophakic RRD cases. (Mean(SD) axial length (known in 49.8%; 440/883) of phakic RRD was 24.81mm(1.71mm); mean(SD) axial length (known in 29.4%; 67/228) of pseudophakic RRD was 24.69mm(1.53mm);p=0.56) (Mean(SD) spherical equivalent refraction (SER) (known in 91.6%;809/883) of phakic RRD was -2.59D(+3.66D); mean(SD) SER (known in 75.9%; 173/228) of pseudophakic RRD was -2.46D(+3.42);p=0.6)

9.2.4 Phakic Status

Overall, 20.2% (228/1130) of cases were pseudophakic at presentation. The vast majority (94.7%-216/228) had phacoemulsification and intra-ocular lens insertion, the remainder having had intra-capsular or extra-capsular cataract extraction. In the younger group without PVD, previous cataract

surgery was relatively uncommon (6.4%, 95%CI=3-11.8%) compared to the group of RRD associated with PVD (23.7%, 95%CI=21.1-26.5%). A higher proportion of pseudophakia (53.3%-8/15) was present in cases of GRT RRD and one (12.5%) of these cases was complicated by vitreous loss.The median time from cataract surgery to presentation with RRD was 3.28 years (IQR= 1.06-7.23 years). Approximately 17% (38/228) of pseudophakic cases had YAG laser capsulotomy, the median time from YAG capsulotomy to presentation with RRD was 2 years (IQR= 0.8-6.1 years). Nineteen percent (44/228) of pseudophakic cases had complicated surgery with posterior capsule rupture noted at the time of cataract surgery. Aphakia was uncommon (<2%) in all incident cases.

9.2.5 Trauma

Overall, trauma was implicated in 10.3% (116/1130; 95%CI= 8.5-12.2%) of all types of RRD (range 7-13%;Table 9.1). Trauma was documented in 55.2% of cases of retinal dialysis RRD explaining the large proportion of young (<40 years) males affected in this group. Head trauma (classified as 'other trauma') was associated with 57.5% (42/73) of PVD associated RRDs and 41.9%(18/43) of non-PVD RRDs in the trauma group. The median time from reported trauma to RRD presentation was 3.48 months with a wide inter-quartile range of between 0.36 to 88.6 months. Lattice degeneration was present in 10.3% (12/116) of traumatic RRD cases.

9.2.6 Predisposing retinal degenerations

Table 9.1 demonstrates the prevalence of predisposing vitreoretinal degenerations in the study group. Overall, lattice degeneration was present in 18.7%(211/1130) of detached eyes, and in 11%(164/1130) of fellow eyes. The fellow eyes of affected eyes with lattice degeneration exhibited

lattice degeneration in 61.6%(130/211). The fellow eyes of PVD associated RRD exhibited lattice degeneration in 13.6% (135/990) which was less than in the fellow eyes of PVD absent RRD (20.7%-29/140; Z=1.97;p=0.049). The highest proportion of fellow eye lattice degeneration was seen in the fellow eyes of RH RRD, where 39.2% (22/56) demonstrated the degeneration.

Lattice degeneration was associated with RH RRD in 35.7%(20/56) of cases compared with 18.9%(188/990) of RRD associated with PVD. (Z=2.56;p=0.01) Similarly, other peripheral retinal degenerations (eg. pavingstone/cobblestone degeneration, peripheral chorio-retinal scarring) were more frequently associated with RH RRD(21.4%-12/56) than RRD associated with PVD(10.4%-103/990) (Z=1.98;p=0.048). In affected eyes with peripheral retinal degeneration other than lattice degeneration, 59% or 79/122 of fellow eyes also demonstrated peripheral retinal degeneration. Lattice degeneration was significantly associated with high myopia; 22.7% (48/211) of eyes with lattice degeneration had a refractive error ≥-6D compared to only 15% (138/919)of eyes without lattice.(Z=2.48; p=0.013) On average eyes with lattice degeneration were 1.4 dioptres more myopic than eyes without.(Eyes without lattice degeneration: Mean(SD) spherical equivalent refraction (D) =-2.23D(3.68); eyes with lattice degeneration: mean(SD) spherical equivalent refractive error= -3.63D(3.62); T=4.77; p<0.0001) This was also reflected in the distribution of fellow eye axial length, where on average, eyes with lattice degeneration had an axial length 0.65mm longer than eyes without lattice degeneration. (Eyes without lattice degeneration: Mean(SD) axial length (mm) =24.65mm(1.70); eyes with lattice degeneration: mean(SD) axial length(mm) = 25.30mm(1.54); T=3.77; p<0.0001) (Figure 9.1)

9.2.7 Familial RRD

Excluding cases of hereditary vitreo-retinopathy, 6.8% (82/1202) of cases reported a first degree relative who also had a rhegmatogenous retinal detachment. The characteristics of this group were: 95%(78/82) were PVD associated RRD, 76 of 82 had a HST RRD, 2 of 82 had a GRT RRD. In 5% (4/82) a PVD was absent; these comprised 3 cases of RH RRD and 1 retinal dialysis. In total, 19.5%(16/82) had lattice degeneration and 17%(14/82) had other peripheral retinal degeneration. Of the sixteen affected eyes with lattice degeneration, 68.7%(11/16) had lattice degeneration in the fellow eye. Similarly, 71.4%(10/14) of affected eyes with other peripheral retinal degeneration demonstrated bilateral disease. The familial group was of similar age and gender distribution to the general RRD population but were on average 1 dioptre more myopic and had an elongated axial length when compared to the general RRD population. (Positive family history group: mean(SD) spherical equivalent refractive error (D) =-3.51(3.61); No family history group: mean(SD) spherical equivalent refractive error (D)= -2.42(3.71); T=2.5;p=0.014) (Figure 9.2)

9.2.7.1 Syndromic RRD

8 cases (0.7%) in the series had ocular and systemic features of syndromic RRD. One case was previously diagnosed with Stickler syndrome. Two further cases exhibited features suggestive of Stickler syndrome: high myopia, an optically empty vitreous, a strong family history of RRD and both cases had GRT RRD. Three cases had systemic developmental features that may be associated with RRD: One case had suspected Pierre-Robin syndrome, one had cleft palate and sensorineural deafness and the third

case had laryngomalacia, developmental delay and hypotonia. One patient was known to have Marfan syndrome and presented with RRD in the context of subluxated lenses. One case known to have Kneist dysplasia presented with a multiple break RRD.

9.3 Discussion

The epidemiological data I present is derived from the Scottish population which is 95.47% White British in ethnicity (http://www.scotland.gov.uk/Publications/2004/02/18876/32939); however the incidence and clinical features of RRD may vary in other populations. The data from this study is, however, the most extensive and detailed ever compiled on the epidemiology of RRD – it provides important clinical information for vitreoretinal surgeons and useful data for healthcare resource planning. For analysis RRDs were divided into those secondary to posterior vitreous separation and tractional (commonly "u") retinal tear development and those without PVD where atrophic (eg round) holes or retinal dialyses were causative. This represents an important clinical distinction as the management of RRD is markedly different depending on posterior vitreous status. The differentiation may also be important in genetic predisposition and pathogenesis of RRD although it is notable that both PVD and non-PVD related RRD are associated with myopia and peripheral retinal degenerations.

In this series, both PVD present and PVD absent RRD were more common in men (M:F 1.5:1-1.61:1). There were, however, notable differences in other associated characteristics between PVD and non-PVD RRDs. RRD associated with PVD demonstrated a higher proportion of right eyes affected (55.2%-95%CI=52-58.3%) and a peak incidence in the 6th decade of life. RRD

without PVD showed no difference in laterality and a peak incidence in the 3rd decade of life. A higher incidence in men, (M:F=1.3:1 to 2.3:1) (Mowatt *et al.* 2003; Ivanisevic *et al.* 2000; Rosman *et al.* 2001; Polkinghorne & Craig 2004b; Limeira-Soares *et al.* 2007) and a predominance of affected right eyes (Right eye:left eye= 1.09:1 to 1.36:1)(Tornquist *et al.* 1987a; Haimann *et al.* 1982a; Laatikainen *et al.* 1985; Li 2003; Rowe *et al.* 1999)have been previously reported in population based studies.

Of the 1,130 cases of primary RRD with detailed clinical information, a PVD associated retinal tear was causative in 87.6% of cases ("u" tears in 86.3%), with non-PVD RRD comprising 12.6% of cases. Non-PVD retinal dialysis was responsible in 5.6% of cases and 55% of these were associated with significant ocular trauma. Complete case ascertainment of GRT can be difficult,(Ang *et al.* 2010) however in our series, GRT and retinoschisis RRD accounted for 1.3% and 1.2% of all cases. The proportion of PVD related RRD in our study is higher than in previously reported clinical series, where HST was the most common causative break in between 50-61% of cases, and atrophic round hole RRD was causative in 12-21% (Laatikainen & Tolppanen 1985b; Sasaki *et al.* 1995; Ashrafzadeh *et al.* 1973; Tornquist *et al.* 1987b) Previous studies have generally classified retinal breaks according to their morphology, so breaks that appear circular may have been treated as atrophic round holes when in fact they were caused by vitreous traction. Alternatively, the higher proportion of PVD related RRD in our series may be due to an older population in Scotland, with a higher number of pseudophakic eyes than in other reports, or it may represent a genuine difference in this population.

High myopia (>-6D) comprised 16.8% of our series in total and was most common in RH and GRT RRD. The proportion of high myopia is similar to

previous European series, where high myopia was present in between 12.7-21.8% of cases(Laatikainen *et al.* 1985; Tornquist *et al.* 1987a), but it is lower than in other Asian populations (32.4% in Beijing, China).(Li 2003) On average RH RRD cases were 1.5 dioptres more myopic than PVD associated RRD cases. It is possible that the Scottish population has a lower incidence of myopia (potentially due to a lesser genetic susceptibility) resulting in fewer myopia-related round-hole RRDs which may result in a higher proportion of PVD related RRDs in our series.

In this population, approximately 1 in 5 presenting cases was pseudophakic, which is comparable to other recent series.(Polkinghorne & Craig 2004b) Pseudophakic RRD was much more common in PVD associated RRD (23.7%-95%CI=21.1-26.5%) than RRD without PVD (6.4%- 95%CI=3-11.8%). Of all pseudophakic cases, 36% had a posterior capsule defect (either due to YAG capsulotomy or complicated cataract surgery), thus approximately two thirds of pseudophakic cases had an intact posterior capsule. The median time to presentation was 3 years post cataract surgery, suggesting that a causal relationship may not apply to all pseudophakic RRDs. No significant difference was noted between the proportions of myopia between phakic and pseudophakic cases. A higher proportion of pseudophakia (53.3%-8/15) was present in cases of GRT RRD. Occurrence of aphakic RRD was rare (<2%) compared with older series,(Ashrafzadeh *et al.* 1973; Everett & Katzin 1968) reflecting modern cataract surgery techniques.

Trauma is an important association of RRD. In Scotland, one in ten cases of primary RRD report ocular or head trauma with a possible temporal relationship to RRD, which is at the upper end of the range reported previously in population based studies (1.6-12.2%).(Sasaki *et al.* 1995; Zou *et al.* 2002; Li 2003; Haimann *et al.* 1982a) The variation in time interval

between injury and presentation (median 3.48 months; IQR=0.36 – 88.56 months) can make it difficult to attribute direct causation of trauma to RRD however the relatively short (3.5 months) median duration between trauma and RRD and the difference in RRD aetiology (a higher proportion of GRTs and retinal dialyses) is evidence that many of these RRDs have a genuine traumatic causation and that non-penetrating trauma may be an underestimated cause of RRD.

Lattice degeneration is a common vitreo-retinal anomaly with a reported population prevalence of 6 to 9.5%.(Byer 1965; Straatsma BR *et al.* 1974) Lattice degeneration was present in 18.7% of all RRDs and in 61% of fellow eyes of these cases, which is within the range of 7-29% reported in previous observational studies from Europe.(Laatikainen & Tolppanen 1985b; Tornquist *et al.* 1987a) In our series, RRD cases with lattice degeneration were significantly more myopic and demonstrated an elongated axial length compared with RRD not associated with the lesion. This suggests that the underlying genetic predisposition to myopia and to lattice degeneration may be linked. Genetic associations of myopia, lattice degeneration and RRD remain, however, uncertain. In Japan, lattice degeneration has been noted in between 60-65% of RRD cases, with refractive error in these cases showing a myopic deviation away from the mean of >2 dioptres.(Sasaki *et al.* 1995)'(Shimizu H 1989) In the Scottish population, all peripheral retinal degenerations including lattice degeneration were more frequently associated with RH RRD compared with PVD associated RRD (21-35% vs 10-19%; $p<0.05$). It was notable also that the incidence of lattice degeneration was similar is trauma associated and non-traumatic RRDs.

A positive first degree relative family history in patients with RRD not associated with other ocular or non-ocular findings has been reported between 1 – 8.2% in large population studies.(Wilkes *et al.* 1982; Zou *et al.* 2002; CUENDET *et al.* 1975) Similarly, our series supports the familial tendency of RRD at the upper end of this range, with approximately 7% of non-syndromic cases reporting an affected first degree relative. This sub-group demonstrated a more myopic refractive error and elongated axial length when compared to the general RRD population.(Figure 9.2) RRD associated with systemic features or hereditary vitreoretinopathy is rare, accounting for 0.7%(8/1130) of our cases, three of these eight cases had features consistent with Stickler syndrome.

In summary, in the Scottish population the proportion of PVD related RRD appears to be higher than previously reported series. RRD cases without a PVD are younger, more myopic and have a higher prevalence of peripheral vitreoretinal degeneration. Lattice degeneration was present in approximately 1 in 5 cases, was frequently bilateral, and associated with higher degrees of myopia. Twenty percent of cases were pseudophakic at presentation, and two thirds of these had had non-complicated cataract surgery. Ocular trauma and familial RRD may be underestimated predispositions occurring in 10% and 7% of cases respectively in this series. Eyes with RRD and associated lattice degeneration or family history of RRD have higher levels of myopia and greater axial lengths than other RRDs.

	PVD PRESENT		PVD ABSENT		
	SINGLE/ MULTIPLE HST N=975(86.3%)	**GRT N=15 (1.3%)**	**ROUND HOLE N=56 (4.9%)**	**DIALYSIS N=67 (5.6%)**	**RETINO-SCHISIS N=17 (1.2%)**
Sex					
Male	598(61.3%)	13(86.7%)	26(46.4%)	44(65.7%)	14(82.3%)
Female	377(38.7%)	2(13.3%)	30(53.6%)	23(35.3%)	3(17.6%)
Age Group					
0-9	0	1(6.7%)	0	1(1.5%)	0
10-19	8(0.8%)	1(6.7%)	3(5.3%)	12(17.9%)	0
20-29	21(2.1%)	0	7(12.5%)	12(17.9%)	0
30-39	48(4.9%)	2(13.3%)	14(25%)	18(26.9%)	0
40-49	107(11%)	1(6.7%)	10(17.8%)	15(22.4%)	0
50-59	263(27%)	3(20%)	8(14.3%)	4(6%)	3(17.6%)
60-69	332(34%)	3(20%)	7(12.5%)	3(4.5%)	5(29.4%)
70-79	149(15.3%)	3(20%)	6(10.7%)	1(1.5%)	8(47%)
80+	47(4.8%)	1(6.7%)	1(1.8%)	1(1.5%)	1(5.9%)
Spherical equivalent refractive error (Dioptres)					
≥-6D	164(16.8%)	4(26.7%)	15(26.8%)	3(4.5%)	0
<-6 to >-1D	349(35.8%)	2(13.3%)	23(41.1%)	11(16.4%)	2(11.8%)
≥-1to ≤+1D	263(27%)	2(13.3%)	7(12.7%)	40(59.7%)	2(11.8%)
>+1to <+6D	78(8%)	3(20%)	3(5.4%)	4(6%)	3(17.6%)
≥+6D	8(0.8%)	0	1(1.8%)	1(1.5%)	4(23.5%)
Not Known	113(11.6%)	4(26.7%)	7(12.5%)	8(11.9%)	4(23.5%)
Affected Eye					
Right	539(55.3%)	8(53.3%)	25(44.6%)	38(56.7%)	6(35.3%)
Left	427(43.8%)	6(40%)	30(53.6%)	24(35.8%)	11(64.7%)
Both	9(0.9%)	1(6.7%)	1(1.8%)	5(7.5%)	0
Phakic status					
Phakia	748(76.5%)	7(46.7%)	51(91.1%)	65(97%)	15(88.2%)
Pseudophakia	213(21.8%)	8(53.3%)	4(7.1%)	1(1.5%)	2(11.8%)
Aphakia	14(1.4%)	0	1(1.8%)	1(1.5%)	0
Trauma	71(7.2%)	2(13.3%)	4(7.1%)	37(55.2%)	2(11.8%)
Blunt	29	2	2	23	0
Other	42	0	2	14	2

Predisposing					
Lattice	188(19.2%)	0	20(35.7%)	3(4.5%)	0
Other	102(10.5%)	1(6.7%)	12(21.4%)	6(8.9%)	1(5.9%)
Vitreous	98(10%)	5(33.3%)	0	5(7.5%)	0

Table 9.1 – Baseline clinical characteristics of 1,130 cases of RRD by type of causative break. N=Number of cases

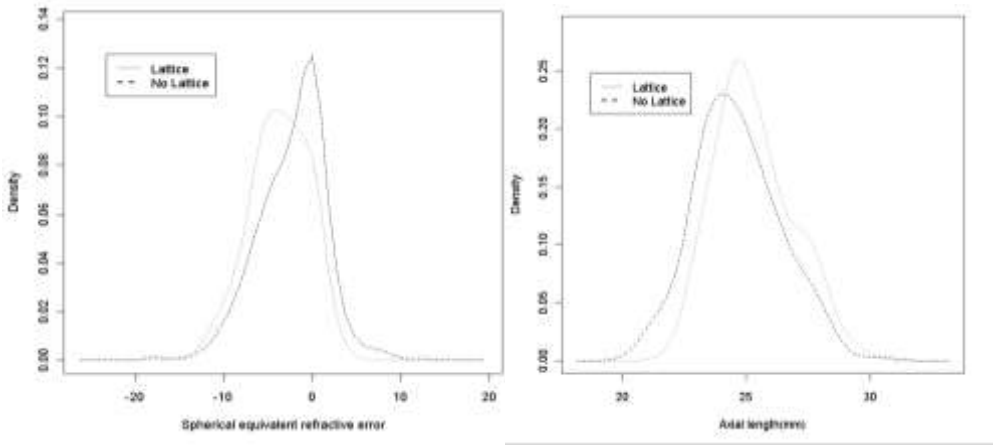


Figure 9.1 – Density plot of the spherical equivalent refractive error(SER) and axial length(AL) of detached eyes with lattice degeneration and those without.(SER was known in 90.5%(191/211) of eyes with lattice degeneration and in 87.8%(807/919) of eyes without)(AL was known in 48.8%(103/211) of eyes with lattice degeneration and in and 43.5%(409/919) of eyes without) Eyes with lattice degeneration were on average 1.3 dioptres more myopic. (Eyes without lattice degeneration: Mean(SD) spherical equivalent refraction (D) =-2.23D(3.68); eyes with lattice degeneration: mean(SD) spherical equivalent refractive error= -3.63D(3.62); T=4.77; p<0.0001) Eyes with lattice degeneration had an average axial length 0.65mm longer than those without. (Eyes without lattice degeneration: Mean(SD) axial length (mm) =24.65mm(1.70); eyes with lattice degeneration: mean(SD) axial length(mm) = 25.30mm(1.54); T=3.77; p<0.0001)

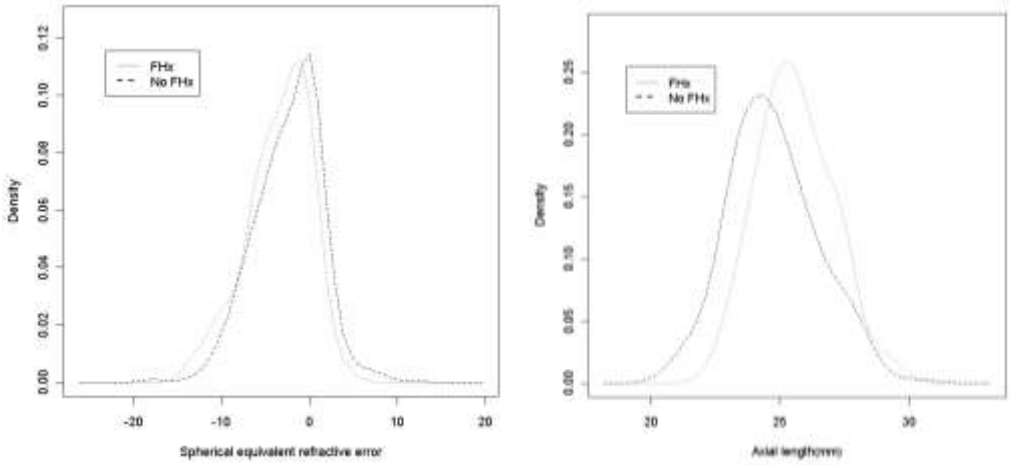


Figure 9.2 – Comparative distribution of spherical equivalent refractive error and axial length of RRD cases with an affected first degree relative and of RRD cases without a family history of the condition.(SER was known in 90.2%(74/82) eyes with a family history of RRD and in 88.2%(924/1048) of eyes without)(AL was known in 39%(32/82) eyes with a family history of RRD and in and 45.8%(480/1048) of eyes without) Excluding cases with known hereditary vitreo-retinopathy, cases with a positive family history of RRD were on average 1 dioptre more myopic. (Positive family history group: mean(SD) spherical equivalent refractive error (D) =-3.51(3.61); No family history group: mean(SD) spherical equivalent refractive error (D)= -2.42(3.71); T=2.5;p=0.014) Similarly, cases with a positive family history

had an average axial length 0.85mm longer than those without. (Positive family history group: mean(SD) axial length (mm) =25.58mm(1.43); No family history group: mean(SD) axial length (mm) = 24.73mm(1.70); T=3.24;p=0.003)

Chapter 10 - Clinical characteristics in the Scottish rhgematogenous retinal detachment Study II – Clinical presentation

10.1 Introduction

Few of the previous epidemiological studies of RRD have described in detail the clinical characteristics of RRD from a large prospectively recruited population. In this section, I aim to describe the clinical features at presentation and the associated ophthalmic pathology in the Scottish RRD Study cases and thus utilize this large population-based RRD cohort to provide clinically important data to aid vitreoretinal surgeons in the assessment and management of RRD.

10.2 Results

A total of 1,244 incident cases of primary RRD were identified in Scotland over the 2 year study period of which 1,202 were recruited to the study and 1,130 had detailed information on clinical features. All cases were classified by the presence or absence of a posterior vitreous detachment and by the type of retinal break(s) causing the detachment. In total, PVD was present in 87.6% (990/1130) of cases; of these cases, single or multiple horse-shoe tear (HST) was causative of RRD in 98.5% (975/990) and giant retinal tear RRD was seen in 1.5% (15/990). PVD was absent in 12.4% (140/1130) of cases and these comprised round hole RRD in 40% (56/140), retinal dialysis in 47.8%(67/140) and retinoschisis RRD in 12.1%(17/140).

Continuous data was analysed using a two-sample T-test and differences in categorical data was analysed using the Z-test of proportionality.

10.2.1 Duration of Symptoms and Route of referral

On presentation, 73.6% (832/1130) of patients reported a peripheral visual field defect, 57.4% (649/1130) floaters and 25.1% (284/1130) photopsia. PVD associated RRD more frequently presented with floaters compared to PVD absent RRD (58%(594/990) vs 39.3%(55/140);Z=4.7;p<0.001), however a similar proportion in both groups presented with phostopsia (25.5% (253/990) vs 22.1%(31/140);Z=0.95;p=0.13) and with a peripheral visual field defect (73.8%(731/990) vs 72.1%(101/140);Z=0.42;p=0.6). Seventy eight percent (879/1130) presented with two or more symptoms, with only 22.2%(251/1130) of cases presenting with a single visual symptom. Less than two percent (15/1130) of cases were asymptomatic, being referred to hospital by an optician/optometrist or primary healthcare worker. The median duration of photopsia was 7 days (IQR=3-21 days), floaters was 10 days (IQR=4-28 days) and of peripheral visual field symptoms was 7 days (IQR= 3-16 days). There was no significant difference in the duration of symptoms between myopes >-1dioptres(D) and non-myopes. The routes of referral differed between myopes (>-1D) and non-myopes. Forty one percent (288/690) of myopes (>-1D) were referred through their optician/optometrist compared to 36.1%(159/440) of non-myopes (Z=1.9;p=0.058). Fifteen percent of non-myopes (67/440) were referred through their general practitioner compared to only 10.2%(70/690) of myopes (Z=2.46;p=0.01). A similar proportion of myopes and non-myopes presented to the emergency services, 20.6% (142/690) and 17.5% (77/440) respectively (Z=1.3;p=0.19). Overall, ninety cases (8%) self referred to hospital.

10.2.2 Intra-ocular pressure (IOP)

We found a significantly lower IOP at presentation in the detached eye compared to the fellow eye in PVD associated RRD, (Mean(SD) IOP in PVD associated RRD =14.7(4.22)mmHg; Mean(SD) IOP in fellow eyes of PVD associated RRD = 15.5(3.48)mmHg; T= 4.02 ;p<0.001). No IOP difference was seen in PVD absent RRD. (Mean(SD) IOP in PVD absent RRD =15.5(5.55)mmHg; Mean(SD) IOP in fellow eyes of PVD absent RRD = 14.7(2.74)mmHg; T= 1.49 ;p=0.14)

10.2.3 Distribution of retinal breaks

The frequency of causative retinal breaks varied with break type. PVD associated HST RRD was caused by single breaks in 57.3% (500/975) and multiple breaks in 48.7% (475/975). In RH RRD there was a significantly higher proportion of multiple breaks with 67.8% (38/56) of cases having more than one causative break. (Z=2.97;p=0.003) The frequency, type and distribution of retinal breaks in detached eyes are shown in table 10.1. Overall, in PVD associated RRD (including GRT), breaks were significantly more frequent in the supero-temporal retina (56.1%- 95%CI=53.8-58.3%), with the second most common location being the superior nasal retina (25.7%- 95%CI=23.7-27.7%). In PVD absent RRD, the site of the causative break in round hole RRD was most commonly infero-temporal (45.9%) or supero-temporal (41.9%). In single break PVD associated RRD cases (excluding GRT RRD) (N=500), 62.2%(311) of cases had a supero-temporal break, 15.6%(78) had a supero-nasal, 16%(80) had an infero-temporal break, and the least common site was infero-nasal in 31 cases(6.2%). A higher distribution of supero-temporal and infero-temporal retinal breaks were found in multiple break PVD associated RRD cases (excluding GRT

RRD) (N=475), where 72.6%(345) of cases had a supero-temporal break, 36.2%(172) had an infero-temporal break, 19.8%(94) had a supero-nasal break and 40 cases(8.4%) had an infero-nasal break. In single break PVD absent RRD (excluding retinoschisis RRD) (N=78),64.1%(50) of cases had an infero-temporal break, 25.6%(20) had a supero-temporal break, six cases had a supero-nasal break and two cases an infero-nasal break. In multiple break PVD absent RRD (excluding retinoschisis RRD) (N=45), 62.2%(28) had an infero-temporal break, 48.8%(22) of cases had a supero-temporal break, three cases had an infero-nasal break and two cases a supero-nasal break. In retinal dialysis and retinoschisis RRD breaks were predominantly infero-temporal accounting for over 72.3% and 62.5% of breaks respectively. Ten percent (7/67) of retinal dialyses had more than one retinal break.

Full-thickness retinal breaks were found in 95/1,130 (8.4%) of fellow eyes on presentation. Of cases with PVD, 8.5% (85/990) had fellow eye breaks, (71.7% (61/85) horse-shoe tears and 28.3%(24/85) round holes) In cases without PVD, fellow eye breaks were present in 7.1%(10/140). Of these, in RH RRD (N=7) all fellow eye breaks were round holes, in dialysis cases (N=3), 1 fellow eye break was a HST, and two fellow eyes also had retinal dialysis.

10.2.4 Previous Retinal prophylaxis

Table 10.1 documents the number of patients who had previous retinal prophylaxis and subsequently had a RRD in the treated eye. Overall, 5.8% (66/1130) of cases had previous treatment aimed at preventing RRD, most commonly laser or cryotherapy to retinal breaks only. Previously treated peripheral retinal degeneration was uncommon. Notably, 3.8% (43/1130) of

fellow eyes had been treated for a retinal break or degeneration and subsequently presented with a RRD in the contra-lateral eye (study eye).

10.2.5 Simultaneous and consecutive bilateral RD

Overall 7.3% (88/1202) of cases had RRD in both eyes- of these, eighteen cases (1.5%) presented with simultaneous bilateral RRD. All cases underwent sequential RRD repair. Of this group of 18 simultaneous bilateral cases; 3 were bilateral HST associated RRD, 4 were bilateral RH RRD, 5 cases had RH RRD in one eye and HST RRD in the fellow eye, 5 cases were bilateral retinal dialysis and one case was a bilateral GRT RRD. Three of five retinal dialysis cases reported significant ocular trauma within 9 months prior to presentation. The proportion of lattice degeneration in simultaneous bilateral RRD (27.8%) was not significantly different from that of the general RRD population. All cases presented with unilateral symptoms and symptom duration did not differ with that of the general RRD population. There was no significant difference in levels of myopia between the eighteen simultaneous bilateral cases and the general RRD population however bilateral cases were significantly younger (mean age =45 years; SD - 18 years; p=0.008).

Seventy cases (5.8%) had had a previous RRD in the contra-lateral eye during or outside the study period. The proportion of lattice degeneration (22.8%-16/70) and myopia >-6 dioptres (24.2%-17/70) in this consecutive bilateral RRD group was similar to the general RRD population, however a greater proportion of these cases (41.4%-29/70) were pseudophakic in the affected eye at presentation compared to the total RRD series(20.2%-228/1130).($Z=3.54; p<0.001$)

10.2.6 Macula Status

The proportion of cases with an attached, bisected or detached macula at presentation is detailed in table 10.2. Across all types of RRD, less than 50% of cases had a fully attached macula at presentation.

10.2.7 Quadrants of Detachment and Proliferative vitreoretinopathy

PVD associated RRD cases presented with up to 2 quadrants of detachment in 66%(95%CI=63-69%), compared to 77.8%%(95%CI- 70-84.4%) in PVD absent RRD;(Z=3.35;p=0.001).(Table 10.2) Total RRD was present in 7.7% (87/1130) of cases. Using a standard classification of proliferative vitreo-retinopathy(PVR) in RRD,(Pastor 1998) pre-operative PVR complicated 11.7%(116/990) of PVD associated RRD and 8.5%(12/140) of PVD absent RRD. This was predominantly grade A or B, however grade C PVR was much more common in PVD associated RRD (22.4%-26/116) compared to PVD absent RRD (0.8%-1/12).

10.2.8 Fellow Eye Best Corrected Visual Acuity

The best corrected visual acuity (BCVA) by type of retinal break in the fellow eye of all RRD cases is shown in table 10.3. Overall, the fellow eyes of most cases had good visual acuity; 79.2% (895/1130) had a BCVA of 6/9 or better, however, 13.1% (148/1130) of affected fellow eyes had a BCVA of 6/18 or worse. In PVD associated RRD the most common causes of BCVA of 6/18 or worse in the fellow eye were: 40%(52/130) cataract, 21.5%(28/130) RRD, 18.4%(24/130) age-related macular degeneration, 9.2%(12/130) amblyopia, 5.3%(7/130) injury and 3%(4/130) primary open angle glaucoma. In PVD absent RRD the most common causes of BCVA of

6/18 or worse in the fellow eye were: 44.4%(8/18) cataract, 22.2%(4/18) injury,16.7%(3/18) RRD and 5.5%(1/18) amblyopia.

10.2.9 Type of Primary Surgery

Table 10.4 highlights the type of primary surgery in 1,130 RRD cases. Significant differences were seen in the type of primary operation performed depending on the type of retinal break. In the presence of a PVD, pars plana vitrectomy(PPV) was the primary procedure in 77.3%(95%CI= 74.5-79.8%) of cases compared to 24.3%(95%CI= 17.4-32.2%) of PVD absent cases. ($Z=13.7$; $p<0.0001$) In the absence of a PVD, an external cryo-buckling procedure (with or without subretinal fluid drainage) was performed in 70% (95%CI= 61.7-77.4%) of cases. Pneumoretinopexy was an uncommon primary procedure in Scotland and was used in approximately 1% of RRD cases.

10.3 Discussion

The clinical presentation of RRD varies primarily in relation to the type, distribution and number of retinal breaks. This has important implications for the clinical management and in particular the selection of surgical procedure for RRD cases. The data presented here from the Scottish RD study highlights key clinical characteristics in the largest prospectively recruited series of RRD where detailed clinical information is available.

The nature of symptoms of RRD can vary markedly and their duration does not reliably indicate the presence, extent or duration of detachment.(Polkinghorne & Craig 2004a; Tanner *et al.* 2000) Notably, in this study, over 75% of cases presented with more than one visual symptom and RRD was asymptomatic in less than two percent of cases. The most

common presenting symptom was a shadow or peripheral visual field defect, present in 73.6% (832/1130) with a median duration of 7 days(IQR=3-16), emphasising that new and relatively acute subjective loss of peripheral vision is an important predictive symptom of RRD.(Hollands *et al.* 2009) There is also a difference in hospital referral patterns between myopes and non-myopes. Myopes (of more than one dioptre) preferentially visited and were referred for treatment by their optician/optometrist, compared to non-myopes who more frequently attended primary care physicians.

The distribution of retinal breaks is an important consideration in the pre-operative assessment and subsequent treatment of RRD. In this series, of PVD associated RRD, 56.1% (95%CI=53.8-58.3%) of breaks were identified in the supero-temporal retina. In non-PVD RRD, 54.6%(95%CI- 47.9-61.1%) of breaks were infero-temporal followed by supero-temporal in 34.9%(95%CI- 28.7-41.5%), however in retinal dialysis and retinoschisis RRD, a higher number of breaks were seen in the infero-temporal quadrant; 72.3%(95%CI=59.8-82.7%) and 62.5%(95%CI=35.4-84.8%) respectively. This finding supports previous observations that between 46.4-57.2% of retinal breaks in older (PVD present) patients and in RH RRD were noted in the supero-temporal retinal quadrant,(Laatikainen & Tolppanen 1985b; Everett & Katzin 1968; Tornquist *et al.* 1987b) and that retinal dialysis was most frequently in the infero-temporal quadrant in between 44.7%(17/38) to 54%(7/13).(Laatikainen & Tolppanen 1985b; Tasman 1972; Zion & Burton 1980)

In the present series, 48.7% of PVD associated RRD and a significantly higher proportion (67.8%) of RH RRD were caused by 2 or more retinal breaks. There are few recent studies on the distribution of retinal breaks in a large RRD population that distinguish between PVD and non-PVD RRD,

however this finding is similar or higher than older reports from clinical series, where 57% of phakic RRD cases (2,351/4,126) (Ashrafzadeh *et al.* 1973), 36%(75/590) [11] of RH RRD and 42%(144/342)(Laatikainen & Tolppanen 1985b) overall were reported to be caused by two or more breaks.(Tornquist *et al.* 1987b) In this series, multiple breaks had a similar retinal quadrant distribution to single break RRD in both PVD present and absent cases. Fellow eye retinal breaks were found in 8.4% of cases, which supports previous estimates of 5-20% and highlights the risk of RRD in the fellow eyes of affected individuals.(Laatikainen 1985; Gupta & Benson 2005) There was no difference in the proportion of fellow eye breaks between PVD present and absent RRD.

Reports of the bilateral incidence of RRD have shown considerable variation (between 9-33%) depending on inclusion criteria and duration of follow up.(Folk & Burton 1982; Gupta & Benson 2005; Krohn & Seland 2000; Michels RG *et al.* 1990) Approximately 2% of the Scottish cases presented with simultaneous bilateral RRD and a further 5.3% of patients had a previous RRD in the contra-lateral eye. Retinal dialyses in younger patients accounted for approximately 30% of simultaneous bilateral cases, with trauma being implicated in 55% of dialysis cases. Notably, a significantly higher proportion (41%) of bilateral RRD cases were pseudophakic on presentation compared to the general RRD population (21.6%), which may reflect a higher risk to the pseudophakic fellow eye in cases of RRD.(Gupta & Benson 2005; Ashrafzadeh *et al.* 1973; Sharma *et al.* 2003)

Involvement of the macula at presentation remains the most significant prognostic indicator of post-operative functional visual outcome after RRD repair.(Pastor *et al.* 2008) Overall less than 50% of all RRD types in Scotland present with the macula fully attached. Referral patterns and

access to vitreoretinal surgical services will clearly influence rates of macular involvement and the figures for this study are broadly similar to those reported in other recent Western studies with macula off detachment rates of 40-60%(Laatikainen & Tolppanen 1985a; Pastor *et al.* 2008; Sullivan PM *et al.* 1997; Rowe *et al.* 1999), compared to a higher rate of 86% in developing countries.(Jalali *et al.* 2005) Thus despite recent advances in RRD treatment, the proportion of cases presenting with a macula-on detachment has not improved dramatically in the last 30 years. (Ashrafzadeh *et al.* 1973)'(Sullivan PM *et al.* 1997; Minihan M. *et al.* 2001)

Previous epidemiology series indicate that between 71-75% of RRD cases present with ≤2 quadrants of detachment.(Laatikainen & Tolppanen 1985b) In our series, a significantly higher proportion of PVD absent RRD (78%) presented with ≤2 quadrants of detachment compared to RRD associated with PVD(66%), suggesting that although PVD related RRD may be more symptomatic its progression is also more rapid. Total retinal detachment was present in approximately 8% of cases at presentation. PVR was present in 11.3%(95%CI= 9.5-13.3%) of all cases and there was no difference in proportions between PVD and non-PVD RRD, however this was mostly early (grade A or B) PVR. More established, grade C, PVR was seen almost exclusively in tractional tear RRDs being present in 2.7% (26/ 975) of cases at presentation.

Laser photocoagulation and cryotherapy are commonly used to treat tractional retinal tears.(Wilkinson 2000) Up to 10% of previously treated tears nevertheless go on to develop RRD.(Mastropasqua *et al.* 1999b) In this series, 5.8% of RRD cases had had retinal prophylaxis to a previously identified retinal break or an area of peripheral retinal degeneration and subsequently detached in the treated eye. In over two thirds of cases, this

treatment was to a retinal break. Similarly, 3.8% of fellow eyes received prophylactic treatment and subsequently detached in the contra-lateral eye (study eye). As I did not record the total number of cases of treated retinal tears in our population it is not possible to comment on the effectiveness of this treatment.

Thirteen percent (148/1130) of affected fellow eyes had a best corrected visual acuity (BCVA) of 6/18 or worse. The most common reason for this was cataract in 40.5%(60/148), however RRD in the fellow eye accounted for 20.9% (31/148) of the reduced visual acuity, highlighting the importance of rhegmatogenous pathology in the other eye.

Pars plana vitrectomy (PPV) was the most common primary surgical procedure performed for all PVD associated cases of RRD (77.3%; 95%CI= 74.5-79.8%). By contrast in PVD absent RRD, external cryo-buckling surgery was performed in 70% (95%CI= 61.7-77.4%) of cases, with PPV being a primary procedure in only 24.3% (95%CI= 17.4-32.2%). Pneumatic retinopexy has previously been recorded to be an uncommon primary procedure for RRD repair in the U.K.(Mastropasqua *et al.* 1999a) and the data from this sudy demonstrate that this remains the case in Scotland.

The Scottish RRD Study has generated extensive and detailed information on a large cohort of retinal detachment patients. The presenting clinical characteristics of the cases in this study provide valuable information for clinicians treating retinal detachments - for example the likelihood of single or multiple retinal breaks and their position. The presenting symptomatology and the incidence of vitreoretinal pathology in the fellow eye are also of value in patient management and in the training of retinal specialists.

	PVD PRESENT		PVD ABSENT		
	SINGLE OR MULTIPLE HST N=975 (86.3%)	**GRT N=15 (1.3%)**	**RH N=56 (4.9%)**	**DIALYSIS N=67 (5.6%)**	**RETINO SCHISIS N=17 (1.2%)**
Proportion of single and multiple breaks in cases					
1 break present	500(51.3%)	14 (93.3%)	18 (32.1%)	60 (89.5%)	6 (35.3%)
2 breaks present	222(22.8%)	1(6.7%)	14(25%)	6(8.9%)	11 (64.7%)
3 breaks present	121(12.5%)	0	8 (14.3%)	1(1.5%)	0
More than 3 breaks present	122(12.6%)	0	16 (28.6%)	0	0
Not Known	10(1%)	0	0	0	0
Number and location of retinal breaks					
Supero-temporal	1069(56%)	11 (57.9%)	62 (41.9%)	13(20%)	5 (31.2%)
Supero-nasal	491(25.7%)	4 (21.1%)	7(4.7%)	4(6.1%)	0
Infero-temporal	251(13.2%)	3 (15.8%)	68 (45.9%)	47 (72.3%)	10 (62.5%)
Infero-nasal	96(5%)	1 (5.26%)	11 (7.4%)	1(1.5%)	1 (6.25%)
Previous retinal prophlaxis in detached eyes of RRD cases					
Yes	57(5.8%)	1(6.7%)	4(7.1%)	2(3%)	2 (11.8%)
Type:					
Laser to periperal degeneration	2	0	0	0	0
Laser to retinal breaks	27	1	3	0	1
Cryo to peripheral degeneration	0	0	0	0	0
Cryo to retinal breaks	12	0	1	0	0
Both laser and cryo to either retinal breaks or degeneration	16	0	0	2	1

Table 10.1 – Highlights the distribution of retinal breaks, the proportion of multiple break RRD in the series and the frequency of cases receiving previous retinal prophylaxis in the detached eye. RH - Round hole; N=Number of cases

	PVD PRESENT		PVD ABSENT		
	SINGLE OR MULTIPLE HST N=975(86.3%)	**GRT N=15(1.3%)**	**ROUND HOLE N=56(4.9%)**	**DIALYSIS N=67(5.6%)**	**RETINOSCHISIS N=17(1.2%)**
Macula					
On	416(42.7%)	7(46.7%)	25(44.6%)	26(38.8%)	6(35.3%)
Bisected	77(7.9%)	0	8(14.3%)	9(13.4%)	4(23.5%)
Off	482(49.4%)	8(53.3%)	23(41.1%)	32(47.8%)	7(41.2%)
Quadrants of Detachment					
1	212(21.7%)	5(33.3%)	12(21.4%)	16(23.9%)	2(11.8%)
2	432(44.3%)	5(33.3%)	30(53.6%)	37(55.2%)	12(70.6%)
3	189(19.4%)	2(13.3%)	8(14.3%)	10(14.9%)	2(11.8%)
4	78(8%)	3(20%)	1(1.8%)	4(6%)	1(5.9%)
Not Known	64(6.6%)	0	5(8.9%)	0	0
PVR					
YES	114(11.7%)	2(13.3%)	5(8.9%)	4(6%)	3(17.7%)
Grade A	46	1	4	2	2
Grade B	42	1	1	2	0
Grade C	26	0	0	0	1

Table 10.2 – The extent of detachment and the proportion of pre-operative PVR in all cases. N=Number of cases

	Fellow Eye Best Corrected Snellen Visual Acuity (Number of cases)									
Type of break in detached eye	**> 6/6**	**6/6**	**6/9**	**6/12**	**6/18**	**6/24**	**6/36**	**6/60**	**CF**	**< CF**
SINGLE OR MULTIPLE HST (N=975) (%)	225 (23.1)	347 (35.6)	196 (20.1)	79 (8.1)	43 (4.4)	16 (1.6)	23 (2.4)	17 (1.7)	9 (0.9)	20 (2.1)
GRT(N=15) (%)	1 (6.7)	7 (46.7)	5 (33.3)	0	2 (13)	0	0	0	0	0
ROUND HOLE (N=56) (%)	20 (35.7)	14 (25)	11 (19.6)	3 (5.4)	2 (3.6)	1 (1.8)	1 (1.8)	1 (1.8)	1 (1.8)	2 (3.6)
DIALYSIS(N=67) (%)	36 (53.7)	13 (19.4)	6 (8.9)	4 (6)	2 (3)	1 (1.5)	1 (1.5)	1 (1.5)	1 (1.5)	2 (3)
RETINOSCHISIS (N=17) (%)	4 (23.5)	7 (41.2)	3 (17.6)	1 (5.9)	0	0	1 (5.9)	0	0	1 (5.9)

Table 10.3 – The distribution of fellow eye best corrected visual acuity by retinal break in all RRD cases.

	PVD PRESENT		**PVD ABSENT**		
	SINGLE OR MULTIPLE HST N=975 (86.3%)	**GRT N=15 (1.3%)**	**ROUND HOLE N=56 (4.9%)**	**DIALYSIS N=67 (5.6%)**	**RETINOSCHISIS N=17 (1.2%)**
Type of Primary Surgery					
Vitrectomy and Tamponade	752(77.1%)	13(86.7%)	19 (33.9%)	5 (7.5%)	10 (58.5%)
External Cryo-Buckle	172(17.6%)	0	34 (60.7%)	59 (88.1%)	5 (29.4%)
Both Vitrectomy and External Buckle	33(3.4%)	2(13.3%)	1(1.8%)	2(3%)	2 (11.8%)
Pneumoretinopexy	7(0.7%)	0	2(3.6%)	1(1.5%)	0
Not operated	5(0.4%)	0	0	0	0
Not Known	6(0.6%)	0	0	0	0

Table 10.4 – The type of RRD and primary surgical procedure. N=Number of cases

CHAPTER 11 - Population based estimate of the sibling recurrence risk ratio for rhegmatogenous retinal detachment

11.1 Introduction

Many epidemiological and genetic studies to date have demonstrated important genetic influences on syndromes associated with RRD and on myopia development.(Young 2009) To date, there are over 15 reported chromosomal regions showing linkage with myopia, the reported range of estimated heritability is between 62-90%(Lee *et al.* 2001; Hammond *et al.* 2001; Lyhne *et al.* 2001) and the sibling recurrence risk ratio for myopia (>-2D) has recently been reported as 2.98 (95%CI=1.56-5.79).(Wojciechowski *et al.* 2005b) Nonetheless, there have been few studies into the heritability of non-syndromic RRD. Population studies indicate that between 1- 8.2% of incident cases of RRD also have an affected first degree relative and recently, the cumulative lifetime risk of RRD has been shown to be 2.6 fold higher in relatives of cases compared to controls.(Wilkes *et al.* 1982; Go *et al.* 2005b; Zou *et al.* 2002) The sibling recurrence risk and its relation to population prevalence (λs) is commonly used in genetic epidemiology to determine the power to detect genetic influences. In this section, I calculate a population based estimate of the sibling recurrence risk ratio for RRD. I also examine the effect of gender, proband age at diagnosis, refractive error, birth order, height and weight on the sibling risk.

11.2 Methods

Of all 1,202 recruited cases, full postal addresses were available for 922 cases. All of these cases were white Caucasian in ethnicity and resident in Scotland. After informed consent, each case was contacted by a postal questionnaire. The questionnaire requested information on a diagnosis of retinal detachment in a relative, the height and weight of the proband, birth order of the index case and the affected relative(s), and the size of the family. (Appendix C) A cover letter was included encouraging return of the questionnaire even if the family history was negative.

11.2.1 Statistical Methods

A widely used measure of familial aggregation is the sibling recurrence-risk ratio (λs), which is defined as the ratio of risk of disease manifestation, given that one's sibling is affected, compared with the disease prevalence in the general population. The λs expresses the increased risk of developing the disease in an individual who has a sibling with the disease, and is a quantifiable measure of the genetic contribution to the disease. (Guo 1998; Khoury *et al.* 1988) Similarly, the parent-offspring recurrence risk ratio is defined as the proportion of affected parent-offspring pairs among all parent-offspring pairs in a population compared with the normal population prevalence and describes the increased risk of developing the disease in an offspring who has a parent with the disease. The normal population prevalence of RRD in Scotland is unfortunately not known and recent high quality data on the prevalence of RRD is lacking. However estimates from the U.S. indicate a normal prevalence in white Caucasians of 0.7%.(Brinton D.A & Wilkinson C.P 2009) I defined the prevalence of RRD as the proportion of individuals in the Scottish population that have had a RRD. Based on the

age standardised incidence of RRD in Scotland(Mitry *et al.* 2010) of 13.09 per 100,000 in men and 7.41 per 100,000 in women and an average life expectancy of 73 years for men and 78 years for women, (http://www.isdscotland.org/isd/files/HLE%20_exec_summary.pdf), I estimated the population prevalence of RRD to be 0.96% (13.09*73/1000) for men and 0.58% for women. The average of these values is 0.77%, thus I estimate that the normal prevalence of RRD in Scotland is expected to be approximately 0.8%.

To calculate the λs parameter, I used a population approach(Olson & Cordell 2000) and the standard formula:

$$\lambda s = Ks/K$$

where Ks is the sibling recurrence risk, denoted as the proportion of affected siblings among all siblings of affecteds in a population and K is the normal population prevalence of the condition.

Using generalized estimating equations (GEE) in a logistic regression model with sibling affected status as the binary outcome, I estimated the odds ratio (OR) of a sibling being affected with RRD given an affected proband and examined the effect of proband characteristics on sibling risk. A repeated measures logistic regression model using GEEs allows the incorporation of covariates and can accommodate for different family sizes.(Farbrother *et al.* 2004) In our model, I used a repeated measures analysis to cluster data by individual family size such that responses from different families are assumed to be independent and responses within families are assumed to be correlated. Regression covariates examined included proband age at diagnosis, gender, spherical equivalent refractive error (SER), lens status, birth order and height. Axial length (AL) measurements were available in

40% of participants but were excluded from the regression analysis due to a high missing rate and fewer data points for regression analysis, however increasing AL was strongly correlated with a more myopic SER (r^2=0.78).

I used stepwise logistic regression to identify the best fitting model with the lowest Akaike Information Criterion (AIC). The AIC is a goodness-of-fit measure of statistical models based upon a likelihood function and is commonly used to select between competing statistical models.(Lindsey & Jones 1998) The main covariates included in the final model were proband age at diagnosis, proband gender and SER. Proband birth order, height and body mass index (BMI) and lens status were not significantly associated in our regression models and were excluded from the final analysis. There were no significant age-gender interaction terms. Table 11.1 highlights the model coefficients and associated standard errors. The statistical analyses were performed using R version 2.1.10 and the software package geepack version 1.0.17.

11.3 Results

In total I received 602 (65.3%) returned questionnaires from index cases that were included in the analysis. The demographic characteristics between respondents and non-respondents were similar: age (respondents- mean(SD) age= 60.4(13) years; non-respondents- mean(SD) age= 58.9(16.7) years); gender (respondents= 57% male; non-respondents= 60.3% male) and spherical equivalent refractive error (SER) (respondents mean(SD) SER= -2.56(3.73)D; non-respondents mean (SD)= -2.49(3.72)D). SER was known for 90% of index cases; 51.8% were myopic

(>-1), 28.1% were emmetropic and 9.6% were hypermetropic(>+1). Of all index cases, 78.2% were phakic, 20.1% were pseudophakic and 1.6% were aphakic at presentation.

Of the 602 families (parents, siblings, offspring), 7.8%(47) had one affected member and 0.5%(3) had two affected members. Including all relatives (grandparents to cousins) 12.1% (73) of probands had a relative affected with RRD and 8 families had two or more affected individuals. In total, 83.2% (501/602) of probands had one or more siblings. Sibship size ranged from 2 to 12 in 501 families, with a mean of 2.5 siblings per family. Table 11.2 and 11.3 highlight the distribution of affected family members and the frequency of affected members by family size.

Logistic regression model coefficients are shown in table 11.1 and are based on all families with at least 2 siblings (N=501). The adjusted OR of a sibling being affected given another affected sibling is 1.91(95%CI: 1.18-3.05) Of covariates examined, the degree of myopia of the proband demonstrated a significant effect on the sibling risk of RRD. An increase of 1 dioptre (D) of myopia in the proband, resulted in a 9.8% increased risk to a sibling to develop RRD. Table 11.4 highlights the increasing odds of RRD in a sibling by the increasing level of myopia in the proband.

Assuming a population prevalence of 0.8%, the sibling recurrence ratio (λs) for RRD is 2.1 (95% CI=1.3-3.2) and the parent-offspring recurrence risk ratio is 2.9 (95%CI – 1.9-4.2). Table 11.5 highlights the sibling recurrence risk ratio for the different classifications of RRD.

Covariate	Beta Coefficient	Standard Error	Wald statistic	P-value
Intercept	-6.2328	1.1575	29	<0.001
SER	-0.0931	0.0437	4.55	0.028
Age	0.0285	0.0161	3.13	0.077
Female	0.4667	0.4835	0.93	0.334
Akaike Information Criterion (AIC): 85.53				

Table 11.1 – The association of RRD affected index case covariates on the sibling risk of RRD among a population of Scottish families. (SER- Spherical equivalent refractive error)

Family members	Number affected with RRD	Total number of family members (including deceased members and half siblings)
Parent		
Father	13	602
Mother	15	602
Paternal		
Grandfather	0	
Aunt	3	850
Uncle	3	808
Cousin	1	2633
Maternal		
Grandfather	4	
Aunt	3	777
Uncle	4	704
Cousin	5	2623
Siblings		
Brother	12	666
Half-brother	0	
Sister	8	590
Half-sister	1	
Offspring		
Son	1	535
Daughter	3	515

Table 11.2 – Highlights the relative distribution of the family members of each index case affected with RRD

Family Size	Number of individuals with RRD (excluding index case)				
	0	**1**	**2**	**3**	**Total**
2	22	3		-	25
3	46	5		-	51
4	86	6	1	-	93
5	106	10		-	116
6	92	9	1	-	102
7	60	6		-	66
8	41	0	1	-	42
9	31	1		-	32
10	15	1		-	16
11	13	0		-	13
12	11	3		-	14
13	6	0		-	6
14	0	2		-	2
15	2	0		-	2
NA	21	1		-	22
Total	552	47	3	-	602

Table 11.3 – Number of families by size (parents, siblings and offspring) and number of cases of RRD (excluding index case) among 3,510 relatives of 602 probands.(NA- Not available)

SER of Proband in dioptres (D)	Odds Ratio (OR) and confidence interval of a sibling being affected with RRD
-1	1.1(1.01-1.20)
-2	1.23(1.1-1.32)
-3	1.37(1.21-1.45)
-4	1.52(1.33-1.59)
-5	1.69(1.46-1.75)
-6	1.87(1.62-2.00)

Table 11.4 – The OR of a sibling developing RRD as a function of the level of myopic equivalent refraction in the affected index case. Adjusting for age and gender, each additional dioptre of myopia in the index case increases the odds of RRD in a sibling by 9.8%. (SER= Spherical equivalent refraction)

	Sibling recurrence risk ratio - λs (95%confidence interval)
All RRD cases	2.1(1.3-3.2)
Male index	1.8(1.0-3.5)
Female index	2.9(1.4-5.1)
Index age ≤60 years	2.29(1-4.3)
Index age >60 years	2.36(1.1-4.1)
Non-myopic RRD	1.9(1.1-3.2)
Myopic RRD (>-1D)	2.8(1.5-4.6)
Phakic RRD only	2.5(1.5-3.9)
Pseudophakic or Aphakic RRD only	2.0(0.4-5.9)

Table 11.5 – The sibling recurrence risk ratio by gender, myopia and lens status of index cases with RRD derived from a Scottish population of 501 sibships.

11.4 Discussion

This data provides evidence that genetics and heritability play an important role in the predisposition to RRD. In 501 Scottish sibships, the risk of having an affected sibling with RRD is increased nearly 2-fold given that one sibling has had the condition. I examined the effect of proband age, gender, height, weight, birth order, lens status and spherical equivalent refraction on the sibling risk. Of these, an increase in spherical equivalent refractive error of 1 dioptre towards myopia in the proband conferred a significant increased risk of RRD to a sibling of 9.8% adjusting for proband age and gender.

This work should however be understood in the context of its limitations. An incomplete questionnaire response rate (65%) may lead to participation bias if persons with an affected sibling are more or less likely to participate. However all cases were encouraged to return the questionnaire regardless of family history and comparison of the demographic characteristics between responders and non-responders did not show significant differences. The diagnosis of RRD in family members was ascertained through a self-reported

questionnaire, which may similarly introduce bias, however RRD is most commonly an acute condition causing visual loss requiring surgical intervention, and thus its' nature is likely to be remembered with a degree of accuracy. Also, the conclusions on the genetic influences of RRD reached by this study depend on inherent assumptions in the design and statistical models used. This demonstration of the familial aggregation of RRD has been derived through contacting all incident cases, thus I cannot comment on the risk of RRD in relatives of unaffected controls. We have sought to model the OR of RRD in a sibling accounting for relevant changes in proband characteristics known from previous epidemiological studies to affect RRD incidence. There may be other shared environmental factors that contribute to the familial aggregation observed and finally, the model I have used has no way of accounting for potential gene-environment interactions.

I estimate the sibling recurrence risk ratio (λs) and the parent offspring recurrence risk ratio of RRD to be 2.1 (95% CI=1.3-3.2) and 2.9 (95%CI – 1.9-4.2) respectively. Hence the estimated prevalence of RRD in siblings of affected cases is twice that of the normal population prevalence (1.68% vs 0.8%). The λs parameter is directly related to the power of genetic studies, however its estimate is subject to bias and is dependent upon the prevalence of affection in the overall population. λs diminishes linearly as the overall population prevalence approaches unity,(Olson & Cordell 2000; Risch 1990) and over or under estimation of population prevalence can thus dramatically affect the estimated λs value. To my knowledge, there have been no previous estimates of the sibling recurrence risk of RRD, however this finding is similar to previous reports of moderate myopia >-2D (2.52 – 95%CI: 1.51-5.87)(Wojciechowski *et al.* 2005a) but less than the reported values for high myopia (reported range: 4.9 - 20).(Farbrother *et al.* 2004;

Guggenheim *et al.* 2000) The λs for phakic RRD is higher than for pseudophakic or aphakic RRD (table 11.5) suggesting that non-genetic factors may have a greater influence on RRD after cataract surgery. The sibling recurrence risk for complex ocular traits with a proven genetic component such as age-related macular degeneration has been demonstrated to be modest (λs or Ks=2.96).(Luo *et al.* 2008) In general however, the higher the λs value (>5), the more likely a significant genetic contribution to the pathogenesis of disease exists although the the relationship between an underlying disease-susceptibility allele and λs is complex.(Rybicki & Elston 2000; Vyse & Todd 1996)

Familial aggregation studies of RRD are uncommon because of the low frequency of the condition, however Go et al(Go *et al.* 2005b) have demonstrated a 3-fold increased frequency of RRD in siblings of affected subjects compared to siblings with unaffected subjects. Accounting for myopia, age and gender did not fully explain the familial aggregation observed. I have similarly noted the odds of having an affected sibling with RRD is significantly increased (OR= 1.91: 95%CI-1.18-3.05) given that one sibling has had the condition. Go et al(Go *et al.* 2005b) report a higher frequency of RRD in relatives of myopic probands and controls compared to non-myopic probands and controls (1.13% vs 0.33%) and interestingly they found a higher frequency of RRD in relatives of myopic probands compared to myopic controls (1.8% vs 0.6%), suggesting that genetic factors other than those controlling myopia may influence RRD pathogenesis but that myopia genes may influence other putative genetic determinants of RRD. The regression analysis similarly demonstrates that increasing degrees of myopia in the proband increases the sibling risk of RRD (by approximately 10% for each spherical equivalent dioptre), but this doesn't completely

account for the familial aggregation observed. The λs for non-myopic RRD is 1.9 (95%CI – 1.1-3.2), which, although lower than the estimate for myopic RRD (2.8 [95% - 1.5 – 4.6]) nonetheless highlights the presence of a genetic component to RRD not associated with myopia.

Myopia and in particular high myopia are one of the most relevant risk factors for RRD development. Several studies have highlighted the importance and the complexity of genetic influences on the etiology of these traits and to date numerous chromosomal regions have been identified through genetic linkage(Naiglin *et al.* 2002; Paluru *et al.* 2003; Young *et al.* 1998) and more recently genome wide association studies.(Nakanishi *et al.* 2009) Marked variability in heritability has been demonstrated between different population ethnicities and the relevance of environmental influences has been confirmed.(Klein *et al.* 2009; Wojciechowski *et al.* 2005c; Saw *et al.* 1999) In a similar way, the genetic influence of RRD is likely to be complex and multi-factorial and may demonstrate similar ethnic variation, as has been noted in population based incidence studies.(Mowatt *et al.* 2003; Rosman *et al.* 2001)

Genetic studies of RRD not associated with known syndromes (such as Stickler syndrome or Wagner syndrome) are in their infancy and few reports confirming linkage to chromosomal regions exist for non-syndromic RRD.(Go *et al.* 2003) Known regions involved in collagen development have been implicated in causing RRD in association with other vitreous abnormalities and may provide potential candidate genes.(Richards *et al.* 2002) Similarly, genes coding for components involved in vitreo-retinal adhesion such as laminin and fibronectin may prove to be important in the pathogenesis of this condition. Despite its limitations, this work which is derived from a large well characterised and stable Caucasian population

highlights that familial occurrence of RRD is a risk factor for its development, proband refractive error significantly influences sibling risk independent of age at diagnosis and gender and that genetic factors are likely to be important in the etiology of non-myopic RRD.

CHAPTER 12 – Genotype Case-Control Association Testing

Quality control procedures and statistical analysis was led and performed primarily by Dr. Veronique Vitart and Dr. Mirna Kirin at the Medical Research Council Human Genetics Unit in Edinburgh. I observed and where possible assisted in the analysis, however I did not directly undertake data manipulation or de novo analysis of the genome wide data.

12.1 Quality control measures

Genotype calling was performed by the Wellcome Trust Clinical Research Facility, Edinburgh using a standard Illumina call algorithm on the genome analysis software BeadStudio and a GenCall cut off of 0.15 and the recommended parameters for the Infinium assay. Genotype call rate for cases and controls was high (>98%).

Quality control analysis was performed in R (v2.10.1), using the GenABEL package. The same thresholds were used for cases and controls. The following per SNP thresholds were used:

Excluded markers out of HWE proportions ($P<1 \times 10^{-10}$) – 1,106 SNPs in control population

Per SNP call rate (<97%) – 8,088 SNPs removed in cases and controls

SNP minor allele frequency(MAF) <0.02 – 9,659 SNPs removed in cases and controls

The following samples were excluded:

Low call rate (<97%) – 20 case samples

High autosomal heterozygosity (False discovery rate (FDR)<1%) – 5 case samples

Whole genome identity-by-state (IBS) sharing (IBS≥0.95) (Figure 11.1) – 13 case samples

Gender discrepancy between phenotypic record and genotype – 4 case samples

There was no overall inflation of the test statistic (λ = 1.0197) in either cases (Case λ(se) = 0.987(0.00019))or controls (Control λ(se) = 1.08(0.00043)). (Figure 12.2)

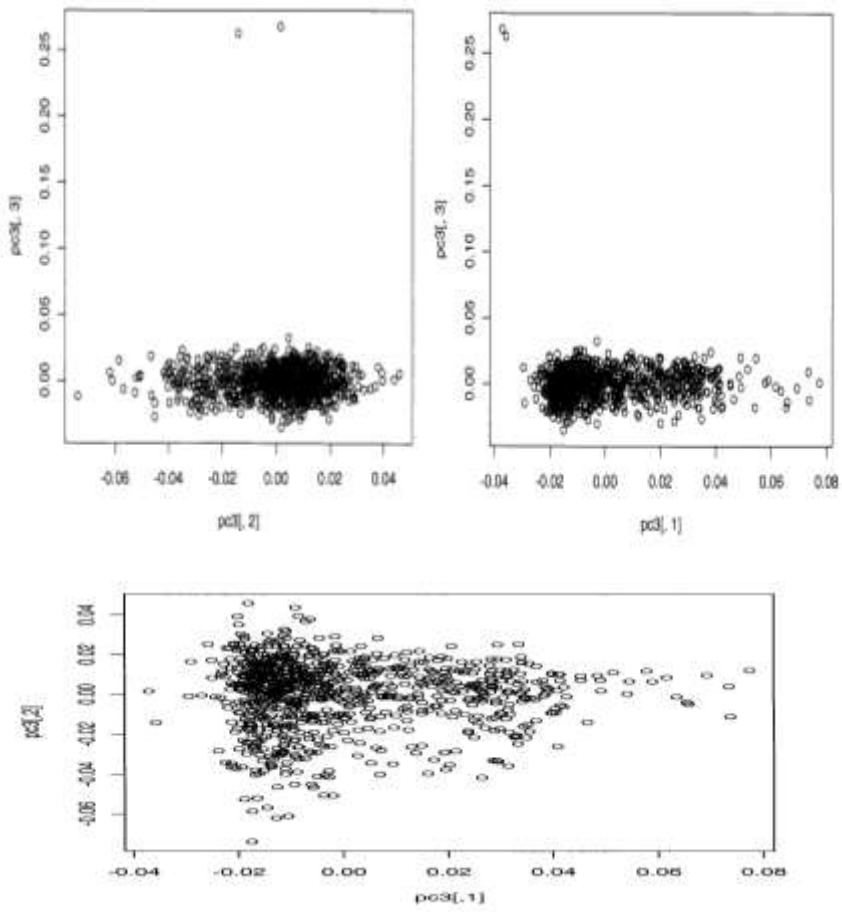


Figure 12.1 – Assessing population stratification by identity by state (IBS) using PLINK and R. Average principal components 1 and 3 and 2 and 3 identified two outlying subjects, an additional 11 outliers were identified outside the graphical display.

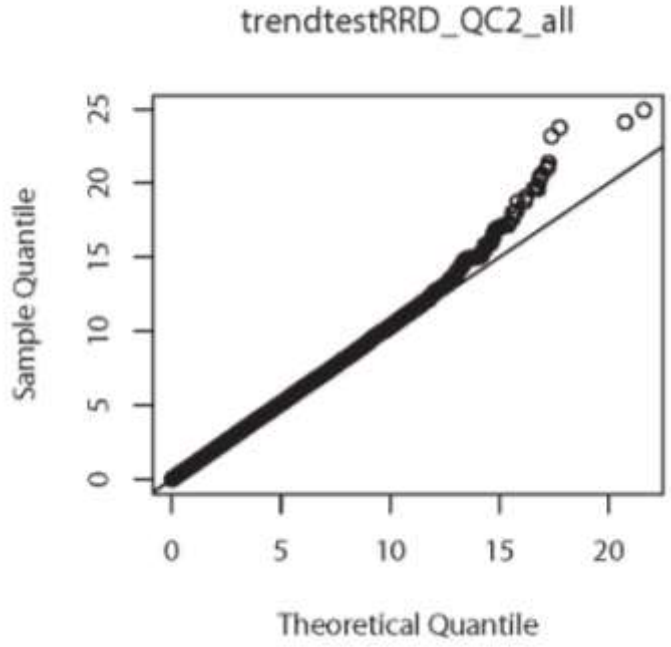


Figure 12.2 – Quantile-Quantile (QQ plot) (excluding poorly clustering SNPs) estimating the unadjusted genomic inflation factor (λ) of cases and controls.

12.2 Association testing

In total after quality control measures, the case-control analysis included 299,869 SNP in 870 cases of RRD and 1,968 controls.

Case control analysis was performed using both PLINK and the GENABEL software in R. A Cochrane-Armitage trend test with 1 degree of freedom was performed with Bonferroni adjusted p-values for genome wide significance. Over 200 SNPs were significant on first analysis. Because of the large number of associated polymorphisms, we suspected an error in the analysis

and compared allele frequencies of these highly associated SNPS in both cases and our controls with previous available genotyped databases (Orkney, Vis, CEU populations on HapMap). We found a large discrepancy between the RRD case genotypes and standard population frequencies and came to the conclusion that clustering for the RRD genotypes using the standard Illumina algorithm was not optimal for the genotypes, and the case genotypes were re-clustered based upon more appropriate thresholds for the genotypic data.

After re-clustering the raw data, the second pass case-control association analyses with Bonferroni correction (p-value $\leq 1.67 \times 10^{-7}$) yielded a total of 14 SNPs reaching genome wide significance. (Table 11.1) The cluster plots of all 14 SNPs were visually inspected using BeadStudio software. In total, only two SNPs showed very clear genotype clusters. The remaining 12 SNPs showed poor clustering in controls (N=2) or in cases (N=10). Two of these 12 poorly clustering SNPs, rs10510663 and rs4679072, showed reasonable genotype clusters and were located in close proximity on chromosome 3 (CLASP2 gene). The two SNPs showing unambiguous and clear clustering, rs4862110 and rs4957798 were very highly associated with the disease phenotype. (Table 12.1 and 12.2)

Chr No.	SNP	Unadjusted p-value	Bonferroni adjusted p-value	Cluster Inspection	Odds Ratio
17	rs7503953	3.16E-274	9.47E-269	Poor case cluster	8.995
16	rs11646140	1.51E-180	4.53E-175	Poor control cluster	9.157
4	rs4862110	4.08E-123	1.22E-117	Good cluster	4.244
19	rs2278902	5.72E-113	1.72E-107	Poor case cluster	10.99
4	rs361147	1.12E-94	3.36E-89	Poor control	0.2075

				cluster	
5	rs4957798	2.46E-86	7.36E-81	Good cluster	3.399
6	rs9500256	6.20E-66	1.86E-60	Poor case cluster	0.2833
4	rs1390266	3.52E-24	1.06E-18	Poor case cluster	2.179
7	rs4722094	1.25E-20	3.76E-15	Poor case cluster	1.85
13	rs1923886	8.34E-13	2.50E-07	Poor case cluster	0.651
20	rs3848693	2.56E-12	7.67E-07	Poor case cluster	1.582
3	rs2319335	2.00E-10	5.99E-05	Poor case cluster	1.499
4	rs10009073	2.12E-10	6.37E-05	Poor case cluster	0.3674
3	rs10510663	3.41E-10	0.000102	Good cluster	1.969
3	rs4679072	3.64E-08	0.01093	Good cluster	0.7194
18	rs2174896	2.45E-07	0.07336	Poor case cluster	0.7337
18	rs9956700	2.94E-07	0.08821	Poor case cluster	0.7248
1	rs1024139	4.40E-07	0.132	Poor case cluster	1.415
2	rs11687980	5.80E-07	0.1739	Poor case cluster	0.6256
18	rs6567038	6.20E-07	0.1859	Poor case cluster	1.476
12	rs11830449	8.35E-07	0.2505	Poor case cluster	0.5879
1	rs10888855	3.35E-06	1	Poor case cluster	1.706

Table 12.1 – Identified polymorphism and corrected p-value for association before covariate adjustment.

Chr No.	SNP	Base Position	Minor Allele	Odds Ratio	Bonferroni Adjusted p-vlaue
17	rs7503953	6082401	A	8.775	1.7E-143
16	rs11646140	29062981	A	9.706	5.5E-115
4	rs4862110	1.84E+08	G	4.088	1.31E-86

4	rs361147	1.53E+08	C	0.2023	8.35E-73
19	rs2278902	55507855	A	11.03	4.93E-68
5	rs4957798	1.08E+08	A	3.499	1.19E-66
6	rs9500256	58416914	A	0.2797	2.25E-52
4	rs1390266	20987259	C	2.061	8.32E-16
7	rs4722094	22098406	G	1.771	2.01E-13
13	rs1923886	46321292	G	0.6533	1.83E-07
20	rs3848693	53866611	A	1.501	1.6E-05
3	rs2319335	1.49E+08	G	1.469	0.000109
4	rs10009073	1.36E+08	G	0.3746	0.000235
3	rs10510663	35534244	G	1.873	0.000385
3	rs4679072	33549244	G	0.7276	0.007878
18	rs2174896	72370842	G	0.7444	0.05445
23	rs12557693	12688352	G	1.562	0.05785

Table 12.2 – SNPs and Bonferroni adjusted p-values after accounting for variables age and gender in the logistic regression analysis.

Covariates of age and gender were added to form a logistic regression model of the case and control genotypes, with disease status as the binary outcome measure. There were significant age and gender effects that diminished the strength of association at the top observed SNP loci. (see Table 12.2)

12.3 Single Nucleotide Polymorphisms - rs4862110 and rs4957798

The two strongest associations after covariate analysis and excluding poor or ambiguous genotype clustering were, rs4862110 and rs4957798, located on chromosomes 4 and 5. Both rs4862110 and rs4957798 were in HWE in both cases (p=0.78 and 0.94) and controls (p=0.45 and 0.15). Case genotype frequencies were 0.47 and 0.5 for the heterozygous C/T genotype for rs4862110 and rs4957798 respectively, compared to control C/T genotype frequencies of 0.28, yielding adjusted p-values for association of 8.02×10^{-109} and 7.46×10^{-81}. Control genotypes were similar to HapMap CEU and other unpublished data (C/T genotype frequency ~0.3).

Because of the large discrepancy noted, we verified these SNP loci in our cases by re-typing them using TaqMan (Applied Biosciences) PCR genotyping. PCR genotyping was performed successfully, there were no false calls and the overall call rate was >98% (Figure 12.3). Table 12.3 highlights the large disagreement in measured allele frequencies for these SNP loci between both technologies.

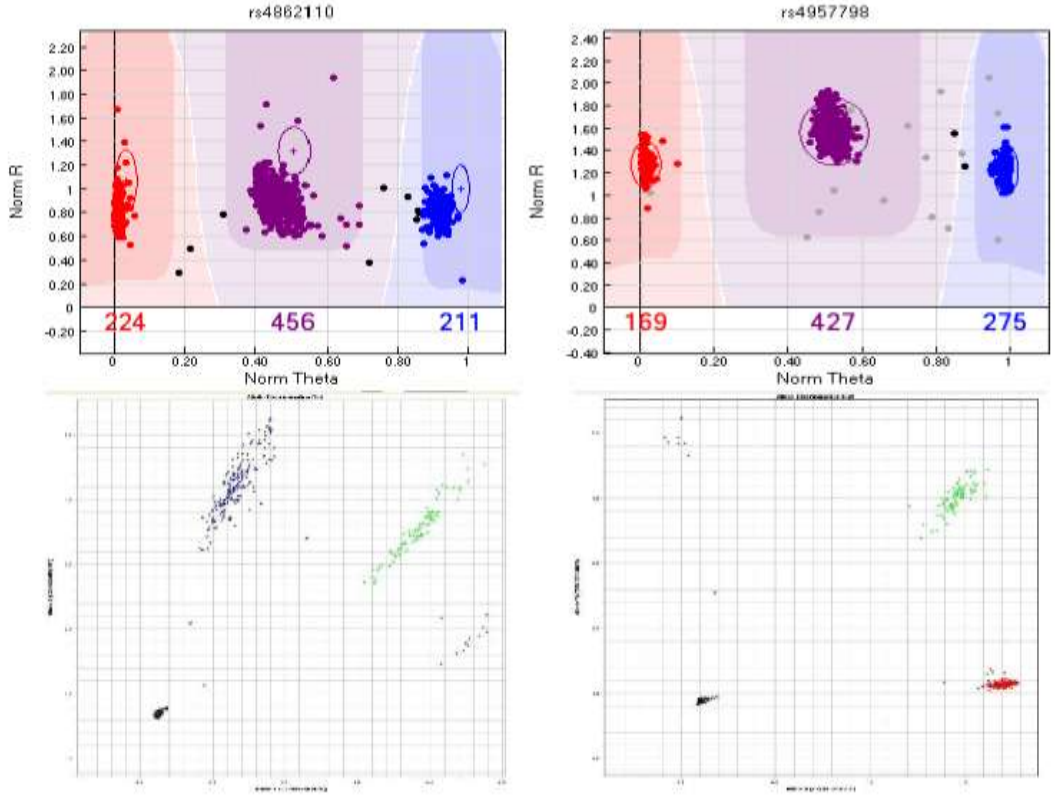


Figure 12.3abcd – Illumina Genomestudio(a+b; top left and top right) and TaqMan PCR(c+d; bottom left and bottom right) allelic discrimination plot of rs4862110 and rs4957798 in our case genotypes.

	rs4862110		**rs4957798**	
	Illumina Call	**TaqMan PCR Call**	**Illumina Call**	**TaqMan PCR Call**
C/C Genotype	211(23.1%)	32(3.5%)	279(30.6%)	588(64.5%)
C/T Genotype	456(50%)	253(27.7%)	436(47.8%)	286(31.4%)
T/T Genotype	224(24.6%)	610(66.9%)	170(18.6%)	26(2.9%)
Undetermined	21(2.3%)	17(1.9%)	27(3%)	12(1.3%)

Table 12.3 – Comparative distribution of measured genotypes for SNPs rs4862110 and rs4957798 using the Illumina 370 Quad array and TaqMan PCR.

Processing of these samples was examined to try and determine why this large discrepancy was noted. There was no sample mis-match, all DNA samples passed standard quality control checks, and there were no array associated processing errors. Both SNP loci genotyped very well, with clear clusters and a high call rate and there was no significant difference in genotypic counts between the 10 processing batches used for the cases (Pearson Goodness of fit test, p=0.302 for rs4862110 and p=0.182 for rs4957798).

I contacted the manufacturers regarding these results and they have advised me that due to new beadpool changes between the Infinium HD and Infinium II products, a small number of loci (<0.01%) appear to show discordant clustering. They recommend excluding the following 31 SNP loci from any analyses using the Human610-quad, 1M-duo and CNV370-Quad version 3 : rs8027714, rs209445, rs9956700, rs1036819, rs10041162, rs2254715, rs1455311, rs2887022, rs3980714, rs6568006, rs2221705, rs7300686, rs9572312, rs12039194, rs10871678, rs1975920, rs7137203, rs2236479, rs11076556, rs1447826, rs2074127, rs3021387, rs12824182, rs6797523, rs777854, rs1549343,rs12582135, rs1810636, rs16883961, rs666101and rs3733746. However, this may not be an exhaustive list as the identified loci were absent and other potentially unidentified SNP variants may demonstrate this discrepancy. This finding emphasises the importance of verifying associated polymorphisms using different genotyping technology prior to further resource investment(McCarthy *et al.* 2008b; McCarthy *et al.* 2008a) and also raises potential compatibility issues when comparing genotypes between newer release platforms and older models by the same manufacturer. Excluding these false positive associations, no individual SNPs reached genome wide significance after stage 1 analysis. (Figure 12.4)

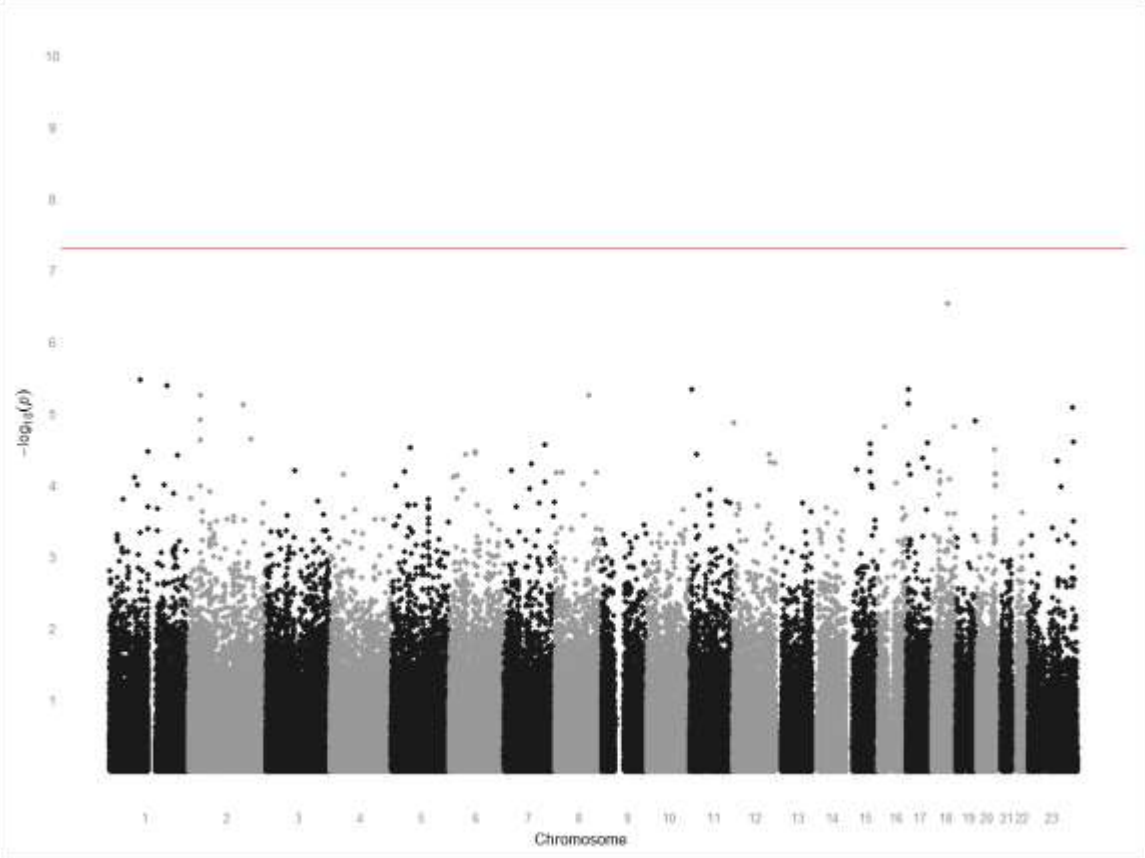


Figure 12.4 - Manhattan plot (excluding poorly clustering SNPs) after adjustment for age and gender

12.4 Imputation

Prediction of missing genotypes at un-typed SNPs in the combined case/control sets was carried out using the IMPUTE program and subsequent analysis carried out via SNPTEST. This was performed by Dr Mirna Kirin. An LD threshold of r^2>0.97 was used. Due to poor concordance between typed and imputed SNPs, we did not select any imputed SNPs for stage 2 replication genotyping. A summary of findings is presented in table 12.4.

SNP	Gene	MAF_RRD (g)	MAF_RRD (i)	Chr	A1	A2	Freq (i)	MAF_socs	chi2_SNP	p
rs4599525	Inter-genic	0.116	0.10	5	C	T	0.8913	0.04	46.01	1.17E-11*
rs6879685	intergenic	0.113	0.10	5	G	T	0.8913	0.04	44.75	2.24E-11*
rs4954847	LRP1B	0.125	0.12	2	A	G	0.1253	0.06	39.64	3.04E-10*
rs4670165	vitrin	0.360	0.36	2	A	C	0.6389	0.42	26.31	2.90E-07*
rs10495897	KCGN3	0.063	0.06	2	C	T	0.9351	0.11	25.88	3.62E-07*
rs10509125		0.392	0.34	10	A	C	0.6569	0.40	21.37	3.77E-06
rs10520735		0.231	0.18	15	A	G	0.8161	0.25	20.28	6.68E-06

Table 12.4 - Statistical summary of imputed case/control SNPs. Asterix highlights SNPs reaching genome wide significance. (g) genotyped; (i) imputed

12.5 Selection of Stage 2 Single Nucleotide Polymorphisms

For stage 2 genotyping we selected the most statistically significant 4,541 SNPs (~1%) from the first stage case control comparison as well as 1,440 SNPs chosen to tag 17 genes (versican/CSPG2, OPTC, LAMC1, LAMB1, LAMA1, KCNJ13, HAS1, HAPLN1, FZD4, FN1, FBN1, COL9A1, COL6A1, COL4A4, COL2A1, COL11A1, COL11A2) that are thought to have a putative biological role in RRD or have been proven to be implicated in syndromic RRD development. In total, 4,235 SNPs were available for analysis in cases (N= 1,072) and controls (N=2,920).

12.5.1 Candidate gene selection

I conducted a thorough literature search on PubMed, Medline, HuGE and Online Mendelian Inheritance in Man(OMIM) for all genes implicated in the pathogenesis of RRD and related syndromes. As well as genes previously associated with the phenotype, I selected genes of known function whose

end products or biological pathways may potentially be involved in RRD pathogenesis. (see Table 12.5) I used the UCSC Genome Browser and the HapMap Geonome Browser to select the genes. The largest RefSeq genes were selected. The genes were visualised and a 10kb region around the gene was examined to determine LD patterns. A force inclusion SNP list was created by selecting known functional coding SNPs in the region and non-synonymous coding SNPs. This list was attached to the tagging algorithm and tagsSNPs were selected for each gene using QuickSNP v1.1, with a MAF of >0.05, r2 of 0.8 and included a 10kb flanking region around the gene. LD was visualised using Haploview with regional genomic data dumps from the HapMap phase II database.

HUGO	**Full/other name**	**Chr**
COL2A1	Collagen II	12q13.11-q13.2
COL4A4	Collagen IV	2q36-q37
COL6A1	Collagen VI	21q22.3
COL9A1	Collagen IX	6q13
COL11A1	STL2/Collagen XI (alpha 1 pp)	1p21
COL11A2	STL3/Collagen XI (alpha 2 pp)	6p21.3
FBN1	Fibrillin 1	15q21.1
FN1	Fibronectin 1	2q34
FZD4	Frizzled Drosophilia Homolog 4	11q14-q21
HAPLN1	Hyaluronan and proteoglycan link protein 1	5q14.3
HAS1	Hyaluronan synthetase 1	19q13.3-q13.4
KCNJ13	SVD/Potassium inwardly-rectifying channel,	2q37

	subfamily J, member 13	
LAMA1	Laminin alpha 1	18p11.31
LAMB1	Laminin beta1	7q31.1-q31.3
LAMC1	Laminin gamma 1	1q31
OPTC	Opticin precursor	1q32.1
VERSICAN/CSPG2	Chondroitin sulfate proteoglycan-2	5q12-q14

Table 12.5 - A summary of the selected candidate genes tagged in stage 2 genotyping

12.6 Association testing - Stage 2

Individually, no SNP reached genome wide significance. A meta analysis of the different cohorts was was performed. Inverse variance weighted fixed and random effects meta-analysis was done using METAL software.(Willer *et al.* 2010) To do this, a reference allele is assigned to each SNP (all studies are aligned to the same reference allele) and its z -statistic is calculated. Based on this an overall z-statistic and p-value are calculated for each SNP from a weighted sum of the individual statistics. Standard errors were used to calculate the effect size weights. After meta analysis of the Dutch (N=264), English (N=687) and Scottish (N=121) replication cohorts, one SNP achieved genome wide statistical significance. (rs11637235, p=1.21E-05) The significance threshold was set at p<1.3e-05 given the numbers of SNPs followed-up for replication, and after correcting for correlated SNPs. rs11637235 is located in chr 15, in the DUT gene, in close proximity to FBN1, which was a selected candidate gene. However this SNP was not significantly associated in the original stage 1 Scottish discovery analysis. A meta analysis of the replication cohorts (Dutch, English and Scottish) together with the

original Scottish RRD cases demonstrated that 5 SNPs reached the significance threshold of 1.3e-05, with one SNP reaching genome wide significance. (Table 12.6) rs12960119 is located in the SS18 gene, which is thought to function as a transcriptional co-activator. It has been found to co-localize with the actin cytoskeleton in cultured cells and RNAi ablation of either isoform markedly impairs the formation of stress fibers and focal adhesions making this a good candidate gene for RRD.(Kim *et al.* 2009)

Chr	SNP	A1	A2	Effect	OR	StdE	P-value	Direction
18	rs12960119	a	g	-0.37	0.69	0.07	1.58E-07	---
1	rs267738	a	c	0.23	1.27	0.05	6.70E-06	+++
4	rs955943	a	g	0.43	1.54	0.09	9.90E-06	+++
10	rs7097067	a	g	0.50	1.65	0.11	1.28E-05	-++
5	rs1074463	a	g	0.26	1.31	0.06	1.28E-05	+++
8	rs2045084	a	g	-0.18	0.83	0.04	1.54E-05	---
21	rs8132771	a	g	0.34	1.43	0.08	1.96E-05	+++

Table 12.6 - Five SNPs achieved the significance threshold, and 1 SNP reached genome wide significance- rs12960119 located within the SS18 gene (p=1.58e-07)

Based on the results from stage 2 meta-analysis, the top 6 statistically significant SNPs were genotyped in an additional 846 samples from London and compared against the genotype counts from 2,737 samples derived from the National Blood Sample control database. rs7097067 was not genotyped as the direction of the effect was not the same in all 3 cohorts. We did not get replication of the genome wide significant SNP (rs12960119), however we did find that

3 SNPs had nominal or close to nominal significant p-value. (0.05)

(Table 12.7 and Figure 12.5)

SNP	A1	A2	BETA	EMPp	EMPp_SE
rs267738	C	A	-0.1706	0.0344	0.0807
rs955943	A	G	0.1904	0.2202	0.1553
rs1074463	A	G	-0.0717	0.4434	0.0935
rs2045084	G	A	0.1269	0.0477	0.0641
rs12960119	G	A	0.1092	0.3312	0.1132
rs8132771	A	G	0.2401	0.0562	0.1257

Table 12.7 - Highlights the empirical p-value of SNPs replicated in an additional 846 cases and 2,737 control samples. One SNP rs8132771 reached close to significance threshold.

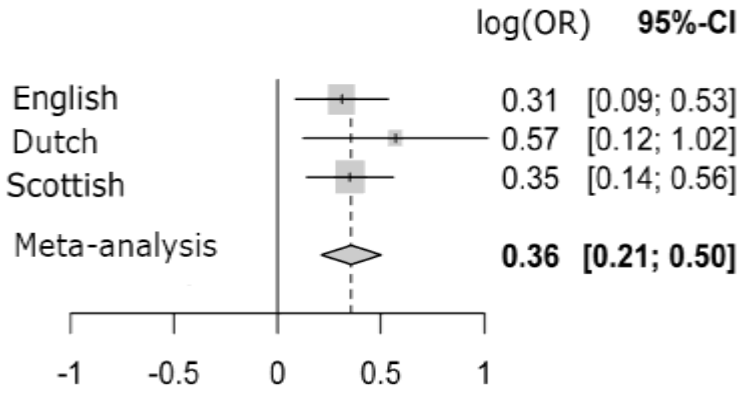


Figure 12.5 - Demonstrates the direction and odds ratio of rs8132771 in all genotyped cohorts.

12.7 Discussion

The three SNPs reaching nominal significance after meta-analysis are: rs267738, rs8132771, rs2045084. rs267738 falls in the gene rich area. rs8132771 falls between TIAM1 gene and SOD1 and rs2045084 tags the TSTA3 gene. At present we are conducting pathway analysis using GRAIL to determine the likelihood of an underlying common pathway.(Raychaudhuri *et al.* 2009) In addition, we will use the software GCTA(Yang *et al.* 2010), to estimate the proportion of the underlying liability's variance that was explained collectively by the variants tested to further demonstrate a genetic basis to RRD risk captured by the SNP array used.

In conclusion, our study was designed to identify common genetic variants of moderate to large effect that may have a role in RRD. We have identified 3 SNPs reaching genome wide significance after meta-analysis. Although our study supports a heritability to RRD, with several interesting genomic regions for further investigation, additional studies of larger sample size will be needed to replicate and verify our findings.

Chapter 13- Conclusions

13.1 Summary of main findings

Prior to my research very little was known about the epidemiology and clinical associations of RRD in the U.K. There had been no previous studies in Scotland and only one report from 2 small regions in England. In published genetic literature, very little was known about the heritability of RRD and there had been no genetic studies on sporadic RRD.

Establishing and maintaning the study presented a significant challenge and necessitated a considerable level of co-operation between clinicians, nurses, healthcare workers and clerical staff. By remaining in constant contact with all six vitreoretinal centres in Scotland I was able to recruit over 90% of all operated cases in Scotland during the study period. One of the great benefits of conducting this work in Scotland was not only the stable population structure, but also the highly developed healthcare service, which facilitated a very effective recruitment process.

My research culminated in the establishment of one of the largest clinical and DNA databases of individuals with primary RRD worldwide. By expanding collaborative efforts with other researchers, I was able to develop active case recruitment in centres elsewhere in the U.K. and in Europe which provided an additional resource for replication of our findings.

There have been many novel findings from this study that have added to our understanding of the nature of RRD. For example, we now know that the annual incidence of primary RRD in the UK is 12.05 per 100,000 population, which equates to over 7,300 new cases each year. Males are more frequently affected in all subtypes and the incidence of RRD shows a strong association with socio-economic affluence despite accounting for known risk

factors. I have described the clinical characteristics and natural history in over 1,200 incident cases of primary RRD, making this one of the most comprehensive reports in published literature. I have reported the frequency of multiple retinal breaks, the association between myopia and non-PVD RRD, the frequency of lattice degeneration by type of RRD and the extent of visual loss in the fellow eye from RRD. My work has established contemporary clinical and demographic associations of RRD which create key clinical distinctions that may have important implications in the underlying pathogenesis of RRD and which are of notable importance in the successful surgical management of patients.

Using the information services division, which manages all hospital episode statistics (HES) in Scotland, I demonstrated that the age standardised incidence of RRD is rising and the average annual percent increase in RRD incidence in Scotland over the 20 years to 2008 was 1.9%. Although commonly this rise in RRD may be attributed to a concomitant rise in cataract surgery in Western countries, I could demonstrate no significant period effect on APC modelling. Examination of the hospital record data from Scotland allowed me to assess and report the high accuracy of case capture by the information services division.

My work was the first in published literature to estimate the genetic contribution to RRD. I have established that the risk to a sibling with RRD is increased two-fold compared to the general population. This risk increases with increasing myopia in the proband, is independent of age and exists for non-myopic RRD. The prevalence of RRD among siblings of affected individuals is twice that of the standard population.

By bringing together researchers from elsewhere in the U.K. and in Europe, I co-ordinated and conducted the first genome wide association study of RRD in 2,833 RRD cases and 7,871 controls in total. Several of the top signals from the analysis have encompassed genes with a documented role in cell adhesion or migration, including SS18, TIAM1, TSTA3 and CDH12. This is the first major insight into the molecular pathogenesis of this complex condition which we hope to investigate further.

13.2 Contribution of this work to the field

My research has highlighted the incidence, clinical characteristics and natural history of RRD in the U.K. This has provided a comprehensive resource which functions to inform clinicians on the subtype and frequency of RRD. These findings will help provide information for future resource allocation and in planning of vitreo-retinal services. I have highlighted a genetic component to RRD pathogenesis. Better characterisation of the genetic risk of RRD will allow more appropriate targeting of prevention measures for individuals as well as for certain population groups. By further exploring the specific genetic loci implicated in RRD, I hope to improve the scientific knowledge of the fundamental molecular basis of RRD. This in turn will afford a potential for rational development of novel agents which may have a role in prevention or treatment of RRD.

13.3 Future research

Having established the largest prospectively recruited database of individuals with primary RRD in the U.K., I am presently performing a national surgical outcome audit to ensure that standards of clinical care in

Scotland are comparable to expected international standards. This clinical database provides the potential for further long term evaluation of the natural history of the condition and for further characterisation of the risk to the second eye. By establishing and expanding our international collaborations, I expect to be in a strong position to replicate our findings in other populations and to move forward to re-sequencing and functional studies to advance our understanding of the biological pathogenesis of this condition.

Appendix A

Checklist of incidence studies

	Score	
1) Definition of RRD	0	No ☐
	1	Yes (Not clear) ☐
	4	Yes (Clear) ☐
2) Type of Study	8	Prospective ☐
	4	ICD coding/Review of Medical Records ☐
	2	Survey or Questionnaire ☐
3) Study Population		
- Demography	1	Present ☐
	0	Absent ☐
- Case selection criteria		
	0	No ☐
	1	Yes (Not clear) ☐
	4	Yes (Clear) ☐
4) Incidence	1	RRD Subtype incidence rates ☐
	1	Age specific incidence rates ☐
	1	Sex specific incidence rates ☐

Maximum score = 20

Appendix B

Retinal Detachment Epidemiology Data Sheet

Centre Number ☐ **Study Number** ☐☐☐☐ **(office use)**

Please complete or tick one option for every question

CHI number: _____ Initials: __ __ Maiden name: _____

Postcode: _____ Tel: _____

Date of Birth: __ __ / __ __ / __ __ __ __

Date of Attendance ___ /___ /___

Sex : Male ☐ Female ☐

Route of referral Self ☐ GP ☐ Optician ☐ A&E ☐ther ☐

Presenting Symptoms	**Yes**	**No**	
Floaters	☐	☐	Duration _____ (days)
Flashes	☐	☐	Duration _____ (days)
Shadow	☐	☐	Duration _____ (days)
Loss of central vision	☐	☐	Duration _____ (days)
Other	_____		Duration _____ (days)

Trauma None ☐ Blunt ☐ Penetrating ☐ Other ☐ specify _____-_____Date ___/___/___

Refraction Myopia ☐ Emmetropia ☐ Hypermetropia ☐ Not known ☐

Axial Length(mm) Right _____ NK ☐ Left _____ NK ☐

Dioptres (Spherical equivalent) Right _____ NK ☐ Left _____ NK ☐

Past Medical History ☐ **Yes**☐ **No** if yes: Diagnosis _____

Ocular History	**Yes**	**No**	
Previous ophthalmic disease	☐	☐	if yes: Diagnosis _____
Previous intraocular surgery	☐	☐	if yes: Type _____ Date ___/___/___
			Type _____ Date ___/___/___
Features of Sticklers	☐	☐	if yes specify:

	Yes	**No**
Sensorineural deafness	☐	☐
Arthropathy	☐	☐
Other	☐	☐

	Yes	**No**	
Complicated cataract Sx with vitreous loss	☐	☐	
YAG	☐	☐	Date ___/___/___

		Yes	**No**		**Yes**	**No**
Previous retinal prophylaxis	Detached eye	☐	☐	Fellow eye	☐	☐
To lattice peripheral retinal degeneration	Cryo	☐	☐	Cryo	☐	☐
	Laser	☐	☐	Laser	☐	☐

To retinal breaks	Cryo	☐	☐	Cryo	☐	☐
	Laser	☐	☐	Laser	☐	☐

Centre Number ☐ **Study Number** ☐☐☐☐ **(office use)**

Family History

	Yes	No	Not Known
FHx of retinal detachment	☐	☐	☐
Members affected: Grandparent	☐	☐	☐
Parent	☐	☐	☐
Sibling	☐	☐	☐
Other relatives	☐	☐	☐
Parental consanguinity	☐	☐	☐

Ethnicity

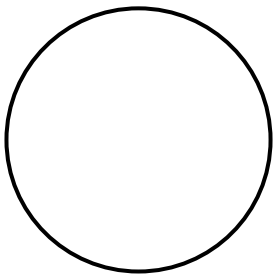
White ☐
Black-African ☐
Black-other ☐
Indian
Pakistani ☐
Chinese ☐
Other ☐
_____

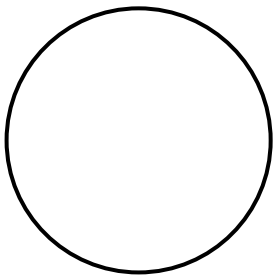
Affected eye **Right** ☐ **Left** ☐

Lens

Phakic	Pseudophakic	Aphakic	Phakic	Pseudophakic	Aphakic
☐	☐	☐	☐	☐	☐

VA (Visual Acuity) **__ / ___** **__ / ___**





Fundus

Macula On ☐ Bisected ☐ Off ☐

Intra-Ocular Pressure (IOP) _____ _____

	Yes	No	Yes	No
Retinal Schisis	☐	☐	☐	☐
Breaks (numbers)	_____		_____	
Round hole	☐	☐	☐	☐
Horse-shoe tear	☐	☐	☐	☐
	☐	☐	☐	☐
Giant tear	☐	☐	☐	☐
Dialysis	☐	☐	☐	☐
Disinsertion				

Yes **No**

PVR ☐ ☐ **Grade** **A** ☐ **B** ☐ **C** ☐

Clock hours of detachment _____ _____

Vitreous	Partially detached	☐	☐	☐	☐
	Detached	☐	☐	☐	☐
	Optically empty	☐	☐	☐	☐
	Haemorrhage	☐	☐	☐	☐
	Membranous deposits	☐	☐	☐	☐
Peripheral retinal degeneration		☐	☐	☐	☐
Posterior lattice degeneration		☐	☐	☐	☐

	Yes	No
Labelled blood sample taken?	☐	☐

Appendix C

Centre Study Number

Dr. Danny Mitry
Department of Public Health
University of Edinburgh
Teviot Place
Edinburgh

Date

Dear

I would like to thank you for taking part in the Scottish Retinal Detachment Study.

Your participation has been immensely valuable and has provided important information about the natural history and epidemiology of retinal detachment which will help in the planning of Ophthalmology services in Scotland.

As part of the study, we are trying to determine how common retinal detachment is in families and if this condition can be inherited. To help inform us, we have attached a brief questionnaire requesting information about the size of your family and if a member of your family has had a retinal detachment. We encourage you to complete the questionnaire, although you may opt out below if you wish. For your reassurance, all the information you provide is stored anonymously in the University of Edinburgh and is used for research purposes only.

We would be very grateful if you could take a moment to complete the attached questionnaire and return it in the stamped self-addressed envelope provided.

Many thanks again for your patience and participation

Best wishes,

_____

Dr. Danny Mitry (Principal Study Co-ordinator)

If you do not wish to complete the questionnaire, please tick this box☐ and return this page.

Centre Study Number

Please complete this page and return it in the stamped envelope provided

	YES	**NO**
	(please tick the box)	
Has anyone in your family had a retinal detachment?	☐	☐

IF YES,

which member(s) were affected:

Father	☐	Mother	☐
Brother	☐	Sister	☐
Half-Brother	☐	Half-Sister	☐
Son	☐	Daughter	☐
Paternal Aunt	☐	Maternal Aunt	☐
Paternal Uncle	☐	Maternal Uncle	☐
Paternal Grandfather	☐	Maternal Grandfather	☐
Paternal 1st Cousin	☐	Maternal 1st Cousin	☐

If a <u>brother or sister</u> have been affected, please indicate their birth order in the family (ie. If they are the first born or eldest, please enter 1. If they are second born or second eldest, please enter 2. If they are third eldest, please enter 3. Etc.) :_____

	<u>Number</u>
In total in your family:	
How many brothers do you have?	_____
How many sisters do you have?	_____
How many sons do you have?	_____
How many daughters do you have?	_____

	Paternal	Maternal
How many Aunts do you have?	_____	_____
How many Uncles do you have?	_____	_____
How many 1st Cousins do you have?	_____	_____

If known, what is your **height** _____ *metres* or ____ *foot/inches* and **weight** _____ *kg* or _____ *stone*

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